TITLE: **HYPERSENSITIVE RESPONSE ELICITING DOMAINS AND USE THEREOF**

INVENTORS: **Hao Fan and Zhong-Min Wei**

DOCKET NO.: **21829/81 (EBC-006)**

HYPERSENSITIVE RESPONSE ELICITING DOMAINS AND USE THEREOF

This application claims benefit of U.S. Provisional Patent Application Serial No. 60/212,211, filed on June 16, 2000.

FIELD OF THE INVENTION

The present invention relates to hypersensitive response elicitors and their structure.

BACKGROUND OF THE INVENTION

Interactions between bacterial pathogens and their plant hosts generally fall into two categories: (1) compatible (pathogen-host), leading to intercellular bacterial growth, symptom development, and disease development in the host plant; and (2) incompatible (pathogen-nonhost), resulting in the hypersensitive response, a particular type of incompatible interaction occurring, without progressive disease symptoms. During compatible interactions on host plants, bacterial populations increase dramatically and progressive symptoms occur. During incompatible interactions, bacterial populations do not increase, and progressive symptoms do not occur.

The hypersensitive response is a rapid, localized necrosis that is associated with the active defense of plants against many pathogens (Kiraly, Z., "Defenses Triggered by the Invader: Hypersensitivity," pages 201-224 in: Plant Disease: An Advanced Treatise, Vol. 5, J.G. Horsfall and E.B. Cowling, ed. Academic Press New York (1980); Klement, Z., "Hypersensitivity," pages 149-177 in: Phytopathogenic Prokaryotes, Vol. 2, M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The hypersensitive response elicited by bacteria is readily observed as a tissue collapse if high concentrations ($\geq 10^7$ cells/ml) of a limited host-range pathogen like *Pseudomonas syringae* or *Erwinia amylovora* are infiltrated into the leaves of nonhost plants (necrosis occurs only in isolated plant cells at lower levels of inoculum) (Klement, Z., "Rapid Detection of Pathogenicity of Phytopathogenic Pseudomonads," Nature 199:299-300; Klement, et al.,

"Hypersensitive Reaction Induced by Phytopathogenic Bacteria in the Tobacco Leaf," Phytopathology 54:474-477 (1963); Turner, et al., "The Quantitative Relation Between Plant and Bacterial Cells Involved in the Hypersensitive Reaction," Phytopathology 64:885-890 (1974); Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York (1982)). The capacities to elicit the hypersensitive response in a nonhost and be pathogenic in a host appear linked. As noted by Klement, Z., "Hypersensitivity," pages 149-177 in Phytopathogenic Prokaryotes, Vol. 2., M.S. Mount and G.H. Lacy, ed. Academic Press, New York, these pathogens also cause physiologically similar, albeit delayed, necroses in their interactions with compatible hosts. Furthermore, the ability to produce the hypersensitive response or pathogenesis is dependent on a common set of genes, denoted *hrp* (Lindgren, P.B., et al., "Gene Cluster of *Pseudomonas syringae* pv. 'phaseolicola' Controls Pathogenicity of Bean Plants and Hypersensitivity on Nonhost Plants," J. Bacteriol. 168:512-22 (1986); Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991)). Consequently, the hypersensitive response may hold clues to both the nature of plant defense and the basis for bacterial pathogenicity.

The *hrp* genes are widespread in gram-negative plant pathogens, where they are clustered, conserved, and in some cases interchangeable (Willis, D.K., et al., "*hrp* Genes of Phytopathogenic Bacteria," Mol. Plant-Microbe Interact. 4:132-138 (1991); Bonas, U., "*hrp* Genes of Phytopathogenic Bacteria," pages 79-98 in: Current Topics in Microbiology and Immunology: Bacterial Pathogenesis of Plants and Animals - Molecular and Cellular Mechanisms, J.L. Dangl, ed. Springer-Verlag, Berlin (1994)). Several *hrp* genes encode components of a protein secretion pathway similar to one used by *Yersinia, Shigella*, and *Salmonella* spp. to secrete proteins essential in animal diseases (Van Gijsegem, et al., "Evolutionary Conservation of Pathogenicity Determinants Among Plant and Animal Pathogenic Bacteria," Trends Microbiol. 1:175-180 (1993)). In *E. amylovora, P. syringae*, and *P. solanacearum*, hrp genes have been shown to control the production and secretion of glycine-rich, protein elicitors of the hypersensitive response (He, S.Y., et al. "Pseudomonas Syringae pv. Syringae HarpinPss: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), Wei, Z.-H.,

et al., "HrpI of *Erwinia amylovora* Functions in Secretion of Harpin and is a Member of a New Protein Family," J. Bacteriol. 175:7958-7967 (1993); Arlat, M. et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-553 (1994)).

The first of these proteins was discovered in *E. amylovora* Ea321, a bacterium that causes fire blight of rosaceous plants, and was designated harpin (Wei, Z.-M., et al, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992)). Mutations in the encoding *hrpN* gene revealed that harpin is required for *E. amylovora* to elicit a hypersensitive response in nonhost tobacco leaves and incite disease symptoms in highly susceptible pear fruit. The *P. solanacearum* GMI1000 PopA1 protein has similar physical properties and also elicits the hypersensitive response in leaves of tobacco, which is not a host of that strain (Arlat, et al. "PopA1, a Protein Which Induces a Hypersensitive-like Response on Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum,*" EMBO J. 13:543-53 (1994)). However, *P. solanacearum popA* mutants still elicit the hypersensitive response in tobacco and incite disease in tomato. Thus, the role of these glycine-rich hypersensitive response elicitors can vary widely among gram-negative plant pathogens.

Other plant pathogenic hypersensitive response elicitors have been isolated, cloned, and sequenced. These include: *Erwinia chrysanthemi* (Bauer, et. al., "*Erwinia chrysanthemi* $Harpin_{Ech}$: Soft-Rot Pathogenesis," MPMI 8(4): 484-91 (1995)); *Erwinia carotovora* (Cui, et. al., "The RsmA$^-$ Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp*N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI 9(7): 565-73 (1966)); *Erwinia stewartii* (Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microb. Inter. July 14-19, 1996 and Ahmad, et. al., "Harpin is not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc. July 27-31, 1996); and *Pseudomonas syringae* pv. *syringae* (WO 94/26782 to Cornell Research Foundation, Inc.).

The present invention is a further advance in the effort to identify and characterize hypersensitive response elicitor proteins.

SUMMARY OF THE INVENTION

One aspect of the present invention is directed to an isolated hypersensitive response elicitor protein comprising a pair of spaced apart domains, with each comprising an acid portion linked to an alpha-helix.

Another embodiment of the present invention relates to an isolated hypersensitive response elicitor protein comprising an acid portion linked to an alpha-helix.

Nucleic acid molecules encoding either of these proteins as well as vectors, host cells, transgenic plants, and transgenic plant seeds containing those nucleic acid molecules are also disclosed.

The protein of the present invention can be used to impart disease resistance to plants, to enhance plant growth, to control insects, and/or impart stress resistance. This involves applying the protein to plants or plant seeds under conditions effective to impart disease resistance, to enhance plant growth, to control insects, and/or impart stress resistance to plants or plants grown from the plant seeds.

As an alternative to applying the protein to plants or plant seeds in order to impart disease resistance, to enhance plant growth, to control insects on plants, and/or impart stress resistance, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a nucleic acid molecule encoding the protein of the present invention and growing the plant under conditions effective to impart disease resistance, to enhance plant growth, to control insects, and/or to impart stress resistance to the plants or plants grown from the plant seeds. Alternatively, a transgenic plant seed transformed with the nucleic acid molecule encoding the protein of the present invention can be provided and planted in soil. A plant is then propagated under conditions effective to impart disease resistance, to enhance plant growth, to control insects, and/or to impart stress resistance to plants or plants grown from the plant seeds.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing showing the construction of a universal expression cassette for a hypersensitive response domain.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to an isolated hypersensitive response elicitor protein comprising a pair of spaced apart domains, with each comprising an acid portion linked to an alpha-helix. The acidic portion is a polypeptide with 10 or more amino acids, is rich in acidic amino acids, and has a pI below 5.0. The acidic portion has a secondary structure in the form of a beta-sheet or a beta-turn. The secondary structure of this unit can also be in an unordered form.

The alpha-helix portion of the present invention is a polypeptide with 10 or more amino acids. Its secondary structure is in the form of a stable alpha-helix.

Another embodiment of the present invention relates to an isolated hypersensitive response elicitor protein comprising an acid portion linked to an alpha-helix.

Both of these proteins are capable of eliciting a hypersensitive response.

The alpha helix is a common structural motif of proteins in which a linear sequence of amino acid folds into a right-handed helix stabilized by internal hydrogen bonding between backbone atoms.

The acidic motif includes a certain combination of amino acids in which a linear sequence with a pI below 5.0 folds into a β sheet, coil, or thin structures but not an alpha helix of secondary structure.

The hypersensitive response elicitor polypeptides or proteins according to the present invention can be derived from hypersensitive response elicitor polypeptides or proteins of a wide variety of fungal and bacterial pathogens. Such polypeptides or proteins are able to elicit local necrosis in plant tissue contacted by the elicitor. Examples of suitable bacterial sources of polypeptide or protein elicitors

include *Erwinia*, *Pseudomonas*, and *Xanthamonas* species (e.g., the following bacteria: *Erwinia amylovora, Erwinia chrysanthemi, Erwinia stewartii, Erwinia carotovora, Pseudomonas syringae, Pseudomonas solancearum, Xanthomonas campestris*, and mixtures thereof). In addition to hypersensitive response elicitors from these Gram negative bacteria, it is possible to use elicitors from Gram positive bacteria. One example is *Clavibacter michiganensis* subsp. *sepedonicus*.

An example of a fungal source of a hypersensitive response elicitor protein or polypeptide is *Phytophthora*. Suitable species of *Phytophthora* include *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamomi, Phytophthora capsici, Phytophthora megasperma*, and *Phytophthora citrophthora*.

The hypersensitive response elicitor polypeptide or protein from *Erwinia chrysanthemi* has an amino acid sequence corresponding to SEQ. ID. No. 1 as follows:

```
Met Gln Ile Thr Ile Lys Ala His Ile Gly Gly Asp Leu Gly Val Ser
1               5                   10                  15

Gly Leu Gly Ala Gln Gly Leu Lys Gly Leu Asn Ser Ala Ala Ser Ser
            20                  25                  30

Leu Gly Ser Ser Val Asp Lys Leu Ser Ser Thr Ile Asp Lys Leu Thr
        35                  40                  45

Ser Ala Leu Thr Ser Met Met Phe Gly Gly Ala Leu Ala Gln Gly Leu
    50                  55                  60

Gly Ala Ser Ser Lys Gly Leu Gly Met Ser Asn Gln Leu Gly Gln Ser
65                  70                  75                  80

Phe Gly Asn Gly Ala Gln Gly Ala Ser Asn Leu Leu Ser Val Pro Lys
                85                  90                  95

Ser Gly Gly Asp Ala Leu Ser Lys Met Phe Asp Lys Ala Leu Asp Asp
            100                 105                 110

Leu Leu Gly His Asp Thr Val Thr Lys Leu Thr Asn Gln Ser Asn Gln
        115                 120                 125

Leu Ala Asn Ser Met Leu Asn Ala Ser Gln Met Thr Gln Gly Asn Met
    130                 135                 140

Asn Ala Phe Gly Ser Gly Val Asn Asn Ala Leu Ser Ser Ile Leu Gly
145                 150                 155                 160

Asn Gly Leu Gly Gln Ser Met Ser Gly Phe Ser Gln Pro Ser Leu Gly
                165                 170                 175
```

```
Ala Gly Gly Leu Gln Gly Leu Ser Gly Ala Gly Ala Phe Asn Gln Leu
            180                 185                 190

Gly Asn Ala Ile Gly Met Gly Val Gly Gln Asn Ala Ala Leu Ser Ala
        195                 200                 205

Leu Ser Asn Val Ser Thr His Val Asp Gly Asn Asn Arg His Phe Val
    210                 215                 220

Asp Lys Glu Asp Arg Gly Met Ala Lys Glu Ile Gly Gln Phe Met Asp
225                 230                 235                 240

Gln Tyr Pro Glu Ile Phe Gly Lys Pro Glu Tyr Gln Lys Asp Gly Trp
                245                 250                 255

Ser Ser Pro Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser Lys
            260                 265                 270

Pro Asp Asp Asp Gly Met Thr Gly Ala Ser Met Asp Lys Phe Arg Gln
        275                 280                 285

Ala Met Gly Met Ile Lys Ser Ala Val Ala Gly Asp Thr Gly Asn Thr
    290                 295                 300

Asn Leu Asn Leu Arg Gly Ala Gly Gly Ala Ser Leu Gly Ile Asp Ala
305                 310                 315                 320

Ala Val Val Gly Asp Lys Ile Ala Asn Met Ser Leu Gly Lys Leu Ala
                325                 330                 335

Asn Ala
```

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34 kDa, is heat stable, has a glycine content of greater than 16%, and contains substantially no cysteine. The *Erwinia chrysanthemi* hypersensitive response elicitor polypeptide or protein is encoded by a DNA molecule having a nucleotide sequence corresponding to SEQ. ID. No. 2 as follows:

```
CGATTTTACC CGGGTGAACG TGCTATGACC GACAGCATCA CGGTATTCGA CACCGTTACG      60

GCGTTTATGG CCGCGATGAA CCGGCATCAG GCGGCGCGCT GGTCGCCGCA ATCCGGCGTC     120

GATCTGGTAT TTCAGTTTGG GGACACCGGG CGTGAACTCA TGATGCAGAT TCAGCCGGGG     180

CAGCAATATC CCGGCATGTT GCGCACGCTG CTCGCTCGTC GTTATCAGCA GGCGGCAGAG     240

TGCGATGGCT GCCATCTGTG CCTGAACGGC AGCGATGTAT TGATCCTCTG GTGGCCGCTG     300

CCGTCGGATC CCGGCAGTTA TCCGCAGGTG ATCGAACGTT TGTTTGAACT GGCGGGAATG     360

ACGTTGCCGT CGCTATCCAT AGCACCGACG GCGCGTCCGC AGACAGGGAA CGGACGCGCC     420

CGATCATTAA GATAAAGGCG GCTTTTTTTA TTGCAAAACG GTAACGGTGA GGAACCGTTT     480
```

CACCGTCGGC GTCACTCAGT AACAAGTATC CATCATGATG CCTACATCGG GATCGGCGTG 540

GGCATCCGTT GCAGATACTT TTGCGAACAC CTGACATGAA TGAGGAAACG AAATTATGCA 600

AATTACGATC AAAGCGCACA TCGGCGGTGA TTTGGGCGTC TCCGGTCTGG GGCTGGGTGC 660

TCAGGGACTG AAAGGACTGA ATTCCGCGGC TTCATCGCTG GGTTCCAGCG TGGATAAACT 720

GAGCAGCACC ATCGATAAGT TGACCTCCGC GCTGACTTCG ATGATGTTTG GCGGCGCGCT 780

GGCGCAGGGG CTGGGCGCCA GCTCGAAGGG GCTGGGGATG AGCAATCAAC TGGGCCAGTC 840

TTTCGGCAAT GGCGCGCAGG GTGCGAGCAA CCTGCTATCC GTACCGAAAT CCGGCGGCGA 900

TGCGTTGTCA AAAATGTTTG ATAAAGCGCT GGACGATCTG CTGGGTCATG ACACCGTGAC 960

CAAGCTGACT AACCAGAGCA ACCAACTGGC TAATTCAATG CTGAACGCCA GCCAGATGAC 1020

CCAGGGTAAT ATGAATGCGT TCGGCAGCGG TGTGAACAAC GCACTGTCGT CCATTCTCGG 1080

CAACGGTCTC GGCCAGTCGA TGAGTGGCTT CTCTCAGCCT TCTCTGGGGG CAGGCGGCTT 1140

GCAGGGCCTG AGCGGCGCGG GTGCATTCAA CCAGTTGGGT AATGCCATCG GCATGGGCGT 1200

GGGGCAGAAT GCTGCGCTGA GTGCGTTGAG TAACGTCAGC ACCCACGTAG ACGGTAACAA 1260

CCGCCACTTT GTAGATAAAG AAGATCGCGG CATGGCGAAA GAGATCGGCC AGTTTATGGA 1320

TCAGTATCCG GAAATATTCG GTAAACCGGA ATACCAGAAA GATGGCTGGA GTTCGCCGAA 1380

GACGGACGAC AAATCCTGGG CTAAAGCGCT GAGTAAACCG GATGATGACG GTATGACCGG 1440

CGCCAGCATG GACAAATTCC GTCAGGCGAT GGGTATGATC AAAAGCGCGG TGGCGGGTGA 1500

TACCGGCAAT ACCAACCTGA ACCTGCGTGG CGCGGGCGGT GCATCGCTGG GTATCGATGC 1560

GGCTGTCGTC GGCGATAAAA TAGCCAACAT GTCGCTGGGT AAGCTGGCCA ACGCCTGATA 1620

ATCTGTGCTG GCCTGATAAA GCGGAAACGA AAAAAGAGAC GGGGAAGCCT GTCTCTTTTC 1680

TTATTATGCG GTTTATGCGG TTACCTGGAC CGGTTAATCA TCGTCATCGA TCTGGTACAA 1740

ACGCACATTT TCCCGTTCAT TCGCGTCGTT ACGCGCCACA ATCGCGATGG CATCTTCCTC 1800

GTCGCTCAGA TTGCGCGGCT GATGGGGAAC GCCGGGTGGA ATATAGAGAA ACTCGCCGGC 1860

CAGATGGAGA CACGTCTGCG ATAAATCTGT GCCGTAACGT GTTTCTATCC GCCCCTTTAG 1920

CAGATAGATT GCGGTTTCGT AATCAACATG GTAATGCGGT TCCGCCTGTG CGCCGGCCGG 1980

GATCACCACA ATATTCATAG AAAGCTGTCT TGCACCTACC GTATCGCGGG AGATACCGAC 2040

AAAATAGGGC AGTTTTTGCG TGGTATCCGT GGGGTGTTCC GGCCTGACAA TCTTGAGTTG 2100

GTTCGTCATC ATCTTTCTCC ATCTGGGCGA CCTGATCGGT T 2141

The hypersensitive response elicitor from *Erwinia chrysanthemi* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ.

ID. No. 1, from amino acid 69 to amino acid 122, particularly from amino acid 85 to amino acid 116. The acidic unit in the first domain extends, within SEQ. ID. No. 1, from amino acid 69 to amino acid 102, particularly from amino acid 85 to amino acid 102. The alpha-helix in the first domain extends, within SEQ. ID. No. 1, from amino acid 102 to amino acid 122, particularly from amino acid 102 to amino acid 116. The second domain extends, within SEQ. ID. No. 1, from amino acid 251 to amino acid 299, particularly from amino acid 256 to amino acid 292. The acidic unit in the second domain extends, within SEQ. ID. No. 1, from amino acid 251 to amino acid 279, particularly from amino acid 261 to amino acid 279. The alpha-helix in the second domain extends, within SEQ. ID. No. 1, from amino acid 279 to amino acid 299, particularly from amino acid 279 to amino acid 292.

The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* has an amino acid sequence corresponding to SEQ. ID. No. 3 as follows:

```
Met Ser Leu Asn Thr Ser Gly Leu Gly Ala Ser Thr Met Gln Ile Ser
1               5                  10                  15

Ile Gly Gly Ala Gly Gly Asn Asn Gly Leu Leu Gly Thr Ser Arg Gln
            20                  25                  30

Asn Ala Gly Leu Gly Gly Asn Ser Ala Leu Gly Leu Gly Gly Gly Asn
        35                  40                  45

Gln Asn Asp Thr Val Asn Gln Leu Ala Gly Leu Leu Thr Gly Met Met
    50                  55                  60

Met Met Met Ser Met Met Gly Gly Gly Gly Leu Met Gly Gly Gly Leu
65                  70                  75                  80

Gly Gly Gly Leu Gly Asn Gly Leu Gly Gly Ser Gly Gly Leu Gly Glu
                85                  90                  95

Gly Leu Ser Asn Ala Leu Asn Asp Met Leu Gly Gly Ser Leu Asn Thr
            100                 105                 110

Leu Gly Ser Lys Gly Gly Asn Asn Thr Thr Ser Thr Thr Asn Ser Pro
        115                 120                 125

Leu Asp Gln Ala Leu Gly Ile Asn Ser Thr Ser Gln Asn Asp Asp Ser
    130                 135                 140

Thr Ser Gly Thr Asp Ser Thr Ser Asp Ser Ser Asp Pro Met Gln Gln
145                 150                 155                 160
```

```
Leu Leu Lys Met Phe Ser Glu Ile Met Gln Ser Leu Phe Gly Asp Gly
                165                 170                 175

Gln Asp Gly Thr Gln Gly Ser Ser Ser Gly Gly Lys Gln Pro Thr Glu
            180                 185                 190

Gly Glu Gln Asn Ala Tyr Lys Lys Gly Val Thr Asp Ala Leu Ser Gly
        195                 200                 205

Leu Met Gly Asn Gly Leu Ser Gln Leu Leu Gly Asn Gly Gly Leu Gly
    210                 215                 220

Gly Gly Gln Gly Gly Asn Ala Gly Thr Gly Leu Asp Gly Ser Ser Leu
225                 230                 235                 240

Gly Gly Lys Gly Leu Gln Asn Leu Ser Gly Pro Val Asp Tyr Gln Gln
                245                 250                 255

Leu Gly Asn Ala Val Gly Thr Gly Ile Gly Met Lys Ala Gly Ile Gln
            260                 265                 270

Ala Leu Asn Asp Ile Gly Thr His Arg His Ser Ser Thr Arg Ser Phe
        275                 280                 285

Val Asn Lys Gly Asp Arg Ala Met Ala Lys Glu Ile Gly Gln Phe Met
    290                 295                 300

Asp Gln Tyr Pro Glu Val Phe Gly Lys Pro Gln Tyr Gln Lys Gly Pro
305                 310                 315                 320

Gly Gln Glu Val Lys Thr Asp Asp Lys Ser Trp Ala Lys Ala Leu Ser
                325                 330                 335

Lys Pro Asp Asp Asp Gly Met Thr Pro Ala Ser Met Glu Gln Phe Asn
            340                 345                 350

Lys Ala Lys Gly Met Ile Lys Arg Pro Met Ala Gly Asp Thr Gly Asn
        355                 360                 365

Gly Asn Leu Gln Ala Arg Gly Ala Gly Gly Ser Ser Leu Gly Ile Asp
    370                 375                 380

Ala Met Met Ala Gly Asp Ala Ile Asn Asn Met Ala Leu Gly Lys Leu
385                 390                 395                 400

Gly Ala Ala
```

This hypersensitive response elicitor polypeptide or protein has a molecular weight of about 39 kDa, has a pI of approximately 4.3, and is heat stable at 100°C for at least 10 minutes. This hypersensitive response elicitor polypeptide or protein has substantially no cysteine. The hypersensitive response elicitor polypeptide or protein derived from *Erwinia amylovora* is more fully described in Wei, Z.-M., R. J. Laby, C. H. Zumoff,

D. W. Bauer, S.-Y. He, A. Collmer, and S. V. Beer, "Harpin, Elicitor of the Hypersensitive Response Produced by the Plant Pathogen *Erwinia amylovora*," Science 257:85-88 (1992), which is hereby incorporated by reference. The DNA molecule encoding this polypeptide or protein has a nucleotide sequence corresponding to SEQ. ID. No. 4 as follows:

```
AAGCTTCGGC ATGGCACGTT TGACCGTTGG GTCGGCAGGG TACGTTTGAA TTATTCATAA      60
GAGGAATACG TTATGAGTCT GAATACAAGT GGGCTGGGAG CGTCAACGAT GCAAATTTCT     120
ATCGGCGGTG CGGGCGGAAA TAACGGGTTG CTGGGTACCA GTCGCCAGAA TGCTGGGTTG     180
GGTGGCAATT CTGCACTGGG GCTGGGCGGC GGTAATCAAA ATGATACCGT CAATCAGCTG     240
GCTGGCTTAC TCACCGGCAT GATGATGATG ATGAGCATGA TGGGCGGTGG TGGGCTGATG     300
GGCGGTGGCT TAGGCGGTGG CTTAGGTAAT GGCTTGGGTG GCTCAGGTGG CCTGGGCGAA     360
GGACTGTCGA ACGCGCTGAA CGATATGTTA GGCGGTTCGC TGAACACGCT GGGCTCGAAA     420
GGCGGCAACA ATACCACTTC AACAACAAAT TCCCCGCTGG ACCAGGCGCT GGGTATTAAC     480
TCAACGTCCC AAAACGACGA TTCCACCTCC GGCACAGATT CCACCTCAGA CTCCAGCGAC     540
CCGATGCAGC AGCTGCTGAA GATGTTCAGC GAGATAATGC AAAGCCTGTT TGGTGATGGG     600
CAAGATGGCA CCCAGGGCAG TTCCTCTGGG GGCAAGCAGC CGACCGAAGG CGAGCAGAAC     660
GCCTATAAAA AAGGAGTCAC TGATGCGCTG TCGGGCCTGA TGGGTAATGG TCTGAGCCAG     720
CTCCTTGGCA ACGGGGGACT GGGAGGTGGT CAGGGCGGTA ATGCTGGCAC GGGTCTTGAC     780
GGTTCGTCGC TGGGCGGCAA AGGGCTGCAA AACCTGAGCG GGCCGGTGGA CTACCAGCAG     840
TTAGGTAACG CCGTGGGTAC CGGTATCGGT ATGAAAGCGG GCATTCAGGC GCTGAATGAT     900
ATCGGTACGC ACAGGCACAG TTCAACCCGT TCTTTCGTCA ATAAAGGCGA TCGGGCGATG     960
GCGAAGGAAA TCGGTCAGTT CATGGACCAG TATCCTGAGG TGTTTGGCAA GCCGCAGTAC    1020
CAGAAAGGCC CGGGTCAGGA GGTGAAAACC GATGACAAAT CATGGGCAAA AGCACTGAGC    1080
AAGCCAGATG ACGACGGAAT GACACCAGCC AGTATGGAGC AGTTCAACAA AGCCAAGGGC    1140
ATGATCAAAA GGCCCATGGC GGGTGATACC GGCAACGGCA ACCTGCAGGC ACGCGGTGCC    1200
GGTGGTTCTT CGCTGGGTAT TGATGCCATG ATGGCCGGTG ATGCCATTAA CAATATGGCA    1260
CTTGGCAAGC TGGGCGCGGC TTAAGCTT                                       1288
```

The hypersensitive response elicitor from *Erwinia amylovora* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID. No. 3, from amino acid 32 to amino acid 74, particularly from amino acid 45 to amino

acid 68. The acidic unit in the first domain extends, within SEQ. ID. No. 3, from amino acid 32 to amino acid 57, particularly from amino acid 45 to amino acid 57. The alpha-helix in the first domain extends, within SEQ. ID. No. 3, from amino acid 57 to amino acid 74, particularly from amino acid 57 to amino acid 68. The second domain extends, within SEQ. ID. No. 3, from amino acid 130 to amino acid 180, particularly from amino acid 145 to amino acid 170. The acidic unit in the second domain extends, within SEQ. ID. No. 3, from amino acid 130 to amino acid 157, particularly from amino acid 145 to amino acid 157. The alpha-helix in the second domain extends, within SEQ. ID. No. 3, from amino acid 157 to amino acid 180, particularly from amino acid 157 to amino acid 170.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,927, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 5 as follows:

```
ATGTCAATTC TTACGCTTAA CAACAATACC TCGTCCTCGC CGGGTCTGTT CCAGTCCGGG      60
GGGGACAACG GGCTTGGTGG TCATAATGCA AATTCTGCGT TGGGGCAACA ACCCATCGAT     120
CGGCAAACCA TTGAGCAAAT GGCTCAATTA TTGGCGGAAC TGTTAAAGTC ACTGCTATCG     180
CCACAATCAG GTAATGCGGC AACCGGAGCC GGTGGCAATG ACCAGACTAC AGGAGTTGGT     240
AACGCTGGCG GCCTGAACGG ACGAAAAGGC ACAGCAGGAA CCACTCCGCA GTCTGACAGT     300
CAGAACATGC TGAGTGAGAT GGGCAACAAC GGGCTGGATC AGGCCATCAC GCCCGATGGC     360
CAGGGCGGCG GGCAGATCGG CGATAATCCT TTACTGAAAG CCATGCTGAA GCTTATTGCA     420
CGCATGATGG ACGGCCAAAG CGATCAGTTT GGCCAACCTG GTACGGGCAA CAACAGTGCC     480
TCTTCCGGTA CTTCTTCATC TGGCGGTTCC CCTTTTAACG ATCTATCAGG GGGGAAGGCC     540
CCTTCCGGCA ACTCCCCTTC CGGCAACTAC TCTCCCGTCA GTACCTTCTC ACCCCCATCC     600
ACGCCAACGT CCCCTACCTC ACCGCTTGAT TTCCCTTCTT CTCCCACCAA AGCAGCCGGG     660
GGCAGCACGC CGGTAACCGA TCATCCTGAC CCTGTTGGTA GCGCGGGCAT CGGGGCCGGA     720
AATTCGGTGG CCTTCACCAG CGCCGGCGCT AATCAGACGG TGCTGCATGA CACCATTACC     780
GTGAAAGCGG GTCAGGTGTT TGATGGCAAA GGACAAACCT TCACCGCCGG TTCAGAATTA     840
GGCGATGGCG GCCAGTCTGA AAACCAGAAA CCGCTGTTTA TACTGGAAGA CGGTGCCAGC     900
CTGAAAAACG TCACCATGGG CGACGACGGG GCGGATGGTA TTCATCTTTA CGGTGATGCC     960
AAAATAGACA ATCTGCACGT CACCAACGTG GGTGAGGACG CGATTACCGT TAAGCCAAAC    1020
AGCGCGGGCA AAAAATCCCA CGTTGAAATC ACTAACAGTT CCTTCGAGCA CGCCTCTGAC    1080
```

```
AAGATCCTGC AGCTGAATGC CGATACTAAC CTGAGCGTTG ACAACGTGAA GGCCAAAGAC    1140
TTTGGTACTT TTGTACGCAC TAACGGCGGT CAACAGGGTA ACTGGGATCT GAATCTGAGC    1200
CATATCAGCG CAGAAGACGG TAAGTTCTCG TTCGTTAAAA GCGATAGCGA GGGGCTAAAC    1260
GTCAATACCA GTGATATCTC ACTGGGTGAT GTTGAAAACC ACTACAAAGT GCCGATGTCC    1320
GCCAACCTGA AGGTGGCTGA ATGA                                           1344
```

See GenBank Accession No. U94513. The isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 6 as follows:

```
Met Ser Ile Leu Thr Leu Asn Asn Asn Thr Ser Ser Ser Pro Gly Leu
1               5                   10                  15

Phe Gln Ser Gly Gly Asp Asn Gly Leu Gly Gly His Asn Ala Asn Ser
            20                  25                  30

Ala Leu Gly Gln Gln Pro Ile Asp Arg Gln Thr Ile Glu Gln Met Ala
        35                  40                  45

Gln Leu Leu Ala Glu Leu Leu Lys Ser Leu Leu Ser Pro Gln Ser Gly
    50                  55                  60

Asn Ala Ala Thr Gly Ala Gly Gly Asn Asp Gln Thr Thr Gly Val Gly
65                  70                  75                      80

Asn Ala Gly Gly Leu Asn Gly Arg Lys Gly Thr Ala Gly Thr Thr Pro
                85                  90                  95

Gln Ser Asp Ser Gln Asn Met Leu Ser Glu Met Gly Asn Asn Gly Leu
            100                 105                 110

Asp Gln Ala Ile Thr Pro Asp Gly Gln Gly Gly Gly Gln Ile Gly Asp
        115                 120                 125

Asn Pro Leu Leu Lys Ala Met Leu Lys Leu Ile Ala Arg Met Met Asp
    130                 135                 140

Gly Gln Ser Asp Gln Phe Gly Gln Pro Gly Thr Gly Asn Asn Ser Ala
145                 150                 155                     160

Ser Ser Gly Thr Ser Ser Ser Gly Gly Ser Pro Phe Asn Asp Leu Ser
                165                 170                 175

Gly Gly Lys Ala Pro Ser Gly Asn Ser Pro Ser Gly Asn Tyr Ser Pro
            180                 185                 190

Val Ser Thr Phe Ser Pro Pro Ser Thr Pro Thr Ser Pro Thr Ser Pro
        195                 200                 205

Leu Asp Phe Pro Ser Ser Pro Thr Lys Ala Ala Gly Gly Ser Thr Pro
    210                 215                 220
```

```
Val Thr Asp His Pro Asp Pro Val Gly Ser Ala Gly Ile Gly Ala Gly
225                 230                 235                 240

Asn Ser Val Ala Phe Thr Ser Ala Gly Ala Asn Gln Thr Val Leu His
                245                 250                 255

Asp Thr Ile Thr Val Lys Ala Gly Gln Val Phe Asp Gly Lys Gly Gln
            260                 265                 270

Thr Phe Thr Ala Gly Ser Glu Leu Gly Asp Gly Gly Gln Ser Glu Asn
        275                 280                 285

Gln Lys Pro Leu Phe Ile Leu Glu Asp Gly Ala Ser Leu Lys Asn Val
    290                 295                 300

Thr Met Gly Asp Asp Gly Ala Asp Gly Ile His Leu Tyr Gly Asp Ala
305                 310                 315                 320

Lys Ile Asp Asn Leu His Val Thr Asn Val Gly Glu Asp Ala Ile Thr
                325                 330                 335

Val Lys Pro Asn Ser Ala Gly Lys Lys Ser His Val Glu Ile Thr Asn
            340                 345                 350

Ser Ser Phe Glu His Ala Ser Asp Lys Ile Leu Gln Leu Asn Ala Asp
        355                 360                 365

Thr Asn Leu Ser Val Asp Asn Val Lys Ala Lys Asp Phe Gly Thr Phe
    370                 375                 380

Val Arg Thr Asn Gly Gly Gln Gln Gly Asn Trp Asp Leu Asn Leu Ser
385                 390                 395                 400

His Ile Ser Ala Glu Asp Gly Lys Phe Ser Phe Val Lys Ser Asp Ser
                405                 410                 415

Glu Gly Leu Asn Val Asn Thr Ser Asp Ile Ser Leu Gly Asp Val Glu
            420                 425                 430

Asn His Tyr Lys Val Pro Met Ser Ala Asn Leu Lys Val Ala Glu
        435                 440                 445
```

This protein or polypeptide is acidic, rich in glycine and serine, and lacks cysteine. It is also heat stable, protease sensitive, and suppressed by inhibitors of plant metabolism. The protein or polypeptide of the present invention has a predicted molecular size of ca. 4.5 kDa.

This hypersensitive response elicitor from *Erwinia amylovora* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID. No. 6, from amino acid 5 to amino acid 64, particularly from amino acid 31 to amino acid 57. The acidic unit in the first domain extends, within SEQ. ID. No. 6, from amino acid 5 to amino acid 45, particularly from amino acid 31 to amino acid 45. The

alpha-helix in the first domain extends, within SEQ. ID. No. 6, from amino acid 45 to amino acid 64, particularly from amino acid 45 to amino acid 64. The second domain extends, within SEQ. ID. No. 6, from amino acid 103 to amino acid 146, particularly from amino acid 116 to amino acid 140. The acidic unit in the second domain extends, within SEQ. ID. No. 6, from amino acid 103 to amino acid 131, particularly from amino acid 116 to amino acid 131. The alpha-helix in the second domain extends, within SEQ. ID. No. 6, from amino acid 131 to amino acid 146, particularly from amino acid 131 to amino acid 140.

Another potentially suitable hypersensitive response elicitor from *Erwinia amylovora* is disclosed in U.S. Patent Application Serial No. 09/120,663, which is hereby incorporated by reference. The protein is encoded by a DNA molecule having a nucleic acid sequence of SEQ. ID. No. 7 as follows:

```
ATGGAATTAA AATCACTGGG AACTGAACAC AAGGCGGCAG TACACACAGC GGCGCACAAC      60
CCTGTGGGGC ATGGTGTTGC CTTACAGCAG GGCAGCAGCA GCAGCAGCCC GCAAAATGCC     120
GCTGCATCAT TGGCGGCAGA AGGCAAAAAT CGTGGGAAAA TGCCGAGAAT TCACCAGCCA     180
TCTACTGCGG CTGATGGTAT CAGCGCTGCT CACCAGCAAA AGAAATCCTT CAGTCTCAGG     240
GGCTGTTTGG GGACGAAAAA ATTTTCCAGA TCGGCACCGC AGGGCCAGCC AGGTACCACC     300
CACAGCAAAG GGGCAACATT GCGCGATCTG CTGGCGCGGG ACGACGGCGA AACGCAGCAT     360
GAGGCGGCCG CGCCAGATGC GGCGCGTTTG ACCCGTTCGG GCGGCGTCAA ACGCCGCAAT     420
ATGGACGACA TGGCCGGGCG GCCAATGGTG AAAGGTGGCA GCGGCGAAGA TAAGGTACCA     480
ACGCAGCAAA AACGGCATCA GCTGAACAAT TTTGGCCAGA TGCGCCAAAC GATGTTGAGC     540
AAAATGGCTC ACCCGGCTTC AGCCAACGCC GGCGATCGCC TGCAGCATTC ACCGCCGCAC     600
ATCCCGGGTA GCCACCACGA AATCAAGGAA GAACCGGTTG GCTCCACCAG CAAGGCAACA     660
ACGGCCCACG CAGACAGAGT GGAAATCGCT CAGGAAGATG ACGACAGCGA ATTCCAGCAA     720
CTGCATCAAC AGCGGCTGGC GCGCGAACGG GAAAATCCAC CGCAGCCGCC CAAACTCGGC     780
GTTGCCACAC CGATTAGCGC CAGGTTTCAG CCCAAACTGA CTGCGGTTGC GGAAAGCGTC     840
CTTGAGGGGA CAGATACCAC GCAGTCACCC CTTAAGCCGC AATCAATGCT GAAAGGAAGT     900
GGAGCCGGGG TAACGCCGCT GGCGGTAACG CTGGATAAAG GCAAGTTGCA GCTGGCACCG     960
GATAATCCAC CCGCGCTCAA TACGTTGTTG AAGCAGACAT TGGGTAAAGA CACCCAGCAC    1020
TATCTGGCGC ACCATGCCAG CAGCGACGGT AGCCAGCATC TGCTGCTGGA CAACAAAGGC    1080
CACCTGTTTG ATATCAAAAG CACCGCCACC AGCTATAGCG TGCTGCACAA CAGCCACCCC    1140
GGTGAGATAA AGGGCAAGCT GGCGCAGGCG GGTACTGGCT CCGTCAGCGT AGACGGTAAA    1200
```

AGCGGCAAGA TCTCGCTGGG GAGCGGTACG CAAAGTCACA ACAAAACAAT GCTAAGCCAA 1260

CCGGGGGAAG CGCACCGTTC CTTATTAACC GGCATTTGGC AGCATCCTGC TGGCGCAGCG 1320

CGGCCGCAGG GCGAGTCAAT CCGCCTGCAT GACGACAAAA TTCATATCCT GCATCCGGAG 1380

CTGGGCGTAT GGCAATCTGC GGATAAAGAT ACCCACAGCC AGCTGTCTCG CCAGGCAGAC 1440

GGTAAGCTCT ATGCGCTGAA AGACAACCGT ACCCTGCAAA ACCTCTCCGA TAATAAATCC 1500

TCAGAAAAGC TGGTCGATAA AATCAAATCG TATTCCGTTG ATCAGCGGGG GCAGGTGGCG 1560

ATCCTGACGG ATACTCCCGG CCGCCATAAG ATGAGTATTA TGCCCTCGCT GGATGCTTCC 1620

CCGGAGAGCC ATATTTCCCT CAGCCTGCAT TTTGCCGATG CCCACCAGGG GTTATTGCAC 1680

GGGAAGTCGG AGCTTGAGGC ACAATCTGTC GCGATCAGCC ATGGGCGACT GGTTGTGGCC 1740

GATAGCGAAG GCAAGCTGTT TAGCGCCGCC ATTCCGAAGC AAGGGGATGG AAACGAACTG 1800

AAAATGAAAG CCATGCCTCA GCATGCGCTC GATGAACATT TTGGTCATGA CCACCAGATT 1860

TCTGGATTTT TCCATGACGA CCACGGCCAG CTTAATGCGC TGGTGAAAAA TAACTTCAGG 1920

CAGCAGCATG CCTGCCCGTT GGGTAACGAT CATCAGTTTC ACCCCGGCTG GAACCTGACT 1980

GATGCGCTGG TTATCGACAA TCAGCTGGGG CTGCATCATA CCAATCCTGA ACCGCATGAG 2040

ATTCTTGATA TGGGGCATTT AGGCAGCCTG GCGTTACAGG AGGGCAAGCT TCACTATTTT 2100

GACCAGCTGA CCAAAGGGTG GACTGGCGCG GAGTCAGATT GTAAGCAGCT GAAAAAAGGC 2160

CTGGATGGAG CAGCTTATCT ACTGAAAGAC GGTGAAGTGA AACGCCTGAA TATTAATCAG 2220

AGCACCTCCT CTATCAAGCA CGGAACGGAA AACGTTTTTT CGCTGCCGCA TGTGCGCAAT 2280

AAACCGGAGC CGGGAGATGC CCTGCAAGGG CTGAATAAAG ACGATAAGGC CCAGGCCATG 2340

GCGGTGATTG GGGTAAATAA ATACCTGGCG CTGACGGAAA AAGGGGACAT TCGCTCCTTC 2400

CAGATAAAAC CCGGCACCCA GCAGTTGGAG CGGCCGGCAC AAACTCTCAG CCGCGAAGGT 2460

ATCAGCGGCG AACTGAAAGA CATTCATGTC GACCACAAGC AGAACCTGTA TGCCTTGACC 2520

CACGAGGGAG AGGTGTTTCA TCAGCCGCGT GAAGCCTGGC AGAATGGTGC CGAAAGCAGC 2580

AGCTGGCACA AACTGGCGTT GCCACAGAGT GAAAGTAAGC TAAAAAGTCT GGACATGAGC 2640

CATGAGCACA AACCGATTGC CACCTTTGAA GACGGTAGCC AGCATCAGCT GAAGGCTGGC 2700

GGCTGGCACG CCTATGCGGC ACCTGAACGC GGGCCGCTGG CGGTGGGTAC CAGCGGTTCA 2760

CAAACCGTCT TTAACCGACT AATGCAGGGG GTGAAAGGCA AGGTGATCCC AGGCAGCGGG 2820

TTGACGGTTA AGCTCTCGGC TCAGACGGGG GGAATGACCG GCGCCGAAGG GCGCAAGGTC 2880

AGCAGTAAAT TTTCCGAAAG GATCCGCGCC TATGCGTTCA ACCCAACAAT GTCCACGCCG 2940

CGACCGATTA AAAATGCTGC TTATGCCACA CAGCACGGCT GGCAGGGGCG TGAGGGGTTG 3000

AAGCCGTTGT ACGAGATGCA GGGAGCGCTG ATTAAACAAC TGGATGCGCA TAACGTTCGT 3060

CATAACGCGC CACAGCCAGA TTTGCAGAGC AAACTGGAAA CTCTGGATTT AGGCGAACAT 3120

GGCGCAGAAT TGCTTAACGA CATGAAGCGC TTCCGCGACG AACTGGAGCA GAGTGCAACC 3180

CGTTCGGTGA CCGTTTTAGG TCAACATCAG GGAGTGCTAA AAAGCAACGG TGAAATCAAT 3240
AGCGAATTTA AGCCATCGCC CGGCAAGGCG TTGGTCCAGA GCTTTAACGT CAATCGCTCT 3300
GGTCAGGATC TAAGCAAGTC ACTGCAACAG GCAGTACATG CCACGCCGCC ATCCGCAGAG 3360
AGTAAACTGC AATCCATGCT GGGGCACTTT GTCAGTGCCG GGGTGGATAT GAGTCATCAG 3420
AAGGGCGAGA TCCCGCTGGG CCGCCAGCGC GATCCGAATG ATAAAACCGC ACTGACCAAA 3480
TCGCGTTTAA TTTTAGATAC CGTGACCATC GGTGAACTGC ATGAACTGGC CGATAAGGCG 3540
AAACTGGTAT CTGACCATAA ACCCGATGCC GATCAGATAA AACAGCTGCG CCAGCAGTTC 3600
GATACGCTGC GTGAAAAGCG GTATGAGAGC AATCCGGTGA AGCATTACAC CGATATGGGC 3660
TTCACCCATA ATAAGGCGCT GGAAGCAAAC TATGATGCGG TCAAAGCCTT TATCAATGCC 3720
TTTAAGAAAG AGCACCACGG CGTCAATCTG ACCACGCGTA CCGTACTGGA ATCACAGGGC 3780
AGTGCGGAGC TGGCGAAGAA GCTCAAGAAT ACGCTGTTGT CCCTGGACAG TGGTGAAAGT 3840
ATGAGCTTCA GCCGGTCATA TGGCGGGGGC GTCAGCACTG TCTTTGTGCC TACCCTTAGC 3900
AAGAAGGTGC CAGTTCCGGT GATCCCCGGA GCCGGCATCA CGCTGGATCG CGCCTATAAC 3960
CTGAGCTTCA GTCGTACCAG CGGCGGATTG AACGTCAGTT TTGGCCGCGA CGGCGGGGTG 4020
AGTGGTAACA TCATGGTCGC TACCGGCCAT GATGTGATGC CCTATATGAC CGGTAAGAAA 4080
ACCAGTGCAG GTAACGCCAG TGACTGGTTG AGCGCAAAAC ATAAAATCAG CCCGGACTTG 4140
CGTATCGGCG CTGCTGTGAG TGGCACCCTG CAAGGAACGC TACAAAACAG CCTGAAGTTT 4200
AAGCTGACAG AGGATGAGCT GCCTGGCTTT ATCCATGGCT TGACGCATGG CACGTTGACC 4260
CCGGCAGAAC TGTTGCAAAA GGGGATCGAA CATCAGATGA AGCAGGGCAG CAAACTGACG 4320
TTTAGCGTCG ATACCTCGGC AAATCTGGAT CTGCGTGCCG GTATCAATCT GAACGAAGAC 4380
GGCAGTAAAC CAAATGGTGT CACTGCCCGT GTTTCTGCCG GGCTAAGTGC ATCGGCAAAC 4440
CTGGCCGCCG GCTCGCGTGA ACGCAGCACC ACCTCTGGCC AGTTTGGCAG CACGACTTCG 4500
GCCAGCAATA ACCGCCCAAC CTTCCTCAAC GGGGTCGGCG CGGGTGCTAA CCTGACGGCT 4560
GCTTTAGGGG TTGCCCATTC ATCTACGCAT GAAGGGAAAC CGGTCGGGAT CTTCCCGGCA 4620
TTTACCTCGA CCAATGTTTC GGCAGCGCTG GCGCTGGATA ACCGTACCTC ACAGAGTATC 4680
AGCCTGGAAT TGAAGCGCGC GGAGCCGGTG ACCAGCAACG ATATCAGCGA GTTGACCTCC 4740
ACGCTGGGAA AACACTTTAA GGATAGCGCC ACAACGAAGA TGCTTGCCGC TCTCAAAGAG 4800
TTAGATGACG CTAAGCCCGC TGAACAACTG CATATTTTAC AGCAGCATTT CAGTGCAAAA 4860
GATGTCGTCG GTGATGAACG CTACGAGGCG GTGCGCAACC TGAAAAAACT GGTGATACGT 4920
CAACAGGCTG CGGACAGCCA CAGCATGGAA TTAGGATCTG CCAGTCACAG CACGACCTAC 4980
AATAATCTGT CGAGAATAAA TAATGACGGC ATTGTCGAGC TGCTACACAA ACATTTCGAT 5040
GCGGCATTAC CAGCAAGCAG TGCCAAACGT CTTGGTGAAA TGATGAATAA CGATCCGGCA 5100

```
CTGAAAGATA TTATTAAGCA GCTGCAAAGT ACGCCGTTCA GCAGCGCCAG CGTGTCGATG    5160

GAGCTGAAAG ATGGTCTGCG TGAGCAGACG GAAAAAGCAA TACTGGACGG TAAGGTCGGT    5220

CGTGAAGAAG TGGGAGTACT TTTCCAGGAT CGTAACAACT TGCGTGTTAA ATCGGTCAGC    5280

GTCAGTCAGT CCGTCAGCAA AAGCGAAGGC TTCAATACCC CAGCGCTGTT ACTGGGGACG    5340

AGCAACAGCG CTGCTATGAG CATGGAGCGC AACATCGGAA CCATTAATTT TAAATACGGC    5400

CAGGATCAGA ACACCCCACG GCGATTTACC CTGGAGGGTG GAATAGCTCA GGCTAATCCG    5460

CAGGTCGCAT CTGCGCTTAC TGATTTGAAG AAGGAAGGGC TGGAAATGAA GAGCTAA       5517
```

This DNA molecule is known as the dspE gene for *Erwinia amylovora*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 8 as follows:

```
Met Glu Leu Lys Ser Leu Gly Thr Glu His Lys Ala Ala Val His Thr
1               5                  10                  15

Ala Ala His Asn Pro Val Gly His Gly Val Ala Leu Gln Gln Gly Ser
            20                  25                  30

Ser Ser Ser Ser Pro Gln Asn Ala Ala Ala Ser Leu Ala Ala Glu Gly
        35                  40                  45

Lys Asn Arg Gly Lys Met Pro Arg Ile His Gln Pro Ser Thr Ala Ala
    50                  55                  60

Asp Gly Ile Ser Ala Ala His Gln Gln Lys Lys Ser Phe Ser Leu Arg
65                  70                  75                  80

Gly Cys Leu Gly Thr Lys Lys Phe Ser Arg Ser Ala Pro Gln Gly Gln
                85                  90                  95

Pro Gly Thr Thr His Ser Lys Gly Ala Thr Leu Arg Asp Leu Leu Ala
            100                 105                 110

Arg Asp Asp Gly Glu Thr Gln His Glu Ala Ala Ala Pro Asp Ala Ala
        115                 120                 125

Arg Leu Thr Arg Ser Gly Gly Val Lys Arg Arg Asn Met Asp Asp Met
    130                 135                 140

Ala Gly Arg Pro Met Val Lys Gly Gly Ser Gly Glu Asp Lys Val Pro
145                 150                 155                 160

Thr Gln Gln Lys Arg His Gln Leu Asn Asn Phe Gly Gln Met Arg Gln
                165                 170                 175

Thr Met Leu Ser Lys Met Ala His Pro Ala Ser Ala Asn Ala Gly Asp
            180                 185                 190

Arg Leu Gln His Ser Pro Pro His Ile Pro Gly Ser His His Glu Ile
        195                 200                 205
```

```
Lys Glu Glu Pro Val Gly Ser Thr Ser Lys Ala Thr Thr Ala His Ala
    210                 215                 220

Asp Arg Val Glu Ile Ala Gln Glu Asp Asp Asp Ser Glu Phe Gln Gln
225                 230                 235                 240

Leu His Gln Gln Arg Leu Ala Arg Glu Arg Glu Asn Pro Pro Gln Pro
                245                 250                 255

Pro Lys Leu Gly Val Ala Thr Pro Ile Ser Ala Arg Phe Gln Pro Lys
            260                 265                 270

Leu Thr Ala Val Ala Glu Ser Val Leu Glu Gly Thr Asp Thr Thr Gln
        275                 280                 285

Ser Pro Leu Lys Pro Gln Ser Met Leu Lys Gly Ser Gly Ala Gly Val
    290                 295                 300

Thr Pro Leu Ala Val Thr Leu Asp Lys Gly Lys Leu Gln Leu Ala Pro
305                 310                 315                 320

Asp Asn Pro Pro Ala Leu Asn Thr Leu Leu Lys Gln Thr Leu Gly Lys
                325                 330                 335

Asp Thr Gln His Tyr Leu Ala His His Ala Ser Ser Asp Gly Ser Gln
            340                 345                 350

His Leu Leu Leu Asp Asn Lys Gly His Leu Phe Asp Ile Lys Ser Thr
        355                 360                 365

Ala Thr Ser Tyr Ser Val Leu His Asn Ser His Pro Gly Glu Ile Lys
    370                 375                 380

Gly Lys Leu Ala Gln Ala Gly Thr Gly Ser Val Ser Val Asp Gly Lys
385                 390                 395                 400

Ser Gly Lys Ile Ser Leu Gly Ser Gly Thr Gln Ser His Asn Lys Thr
                405                 410                 415

Met Leu Ser Gln Pro Gly Glu Ala His Arg Ser Leu Leu Thr Gly Ile
            420                 425                 430

Trp Gln His Pro Ala Gly Ala Ala Arg Pro Gln Gly Glu Ser Ile Arg
        435                 440                 445

Leu His Asp Asp Lys Ile His Ile Leu His Pro Glu Leu Gly Val Trp
    450                 455                 460

Gln Ser Ala Asp Lys Asp Thr His Ser Gln Leu Ser Arg Gln Ala Asp
465                 470                 475                 480

Gly Lys Leu Tyr Ala Leu Lys Asp Asn Arg Thr Leu Gln Asn Leu Ser
                485                 490                 495

Asp Asn Lys Ser Ser Glu Lys Leu Val Asp Lys Ile Lys Ser Tyr Ser
            500                 505                 510

Val Asp Gln Arg Gly Gln Val Ala Ile Leu Thr Asp Thr Pro Gly Arg
        515                 520                 525

His Lys Met Ser Ile Met Pro Ser Leu Asp Ala Ser Pro Glu Ser His
    530                 535                 540

Ile Ser Leu Ser Leu His Phe Ala Asp Ala His Gln Gly Leu Leu His
545                 550                 555                 560
```

```
Gly Lys Ser Glu Leu Glu Ala Gln Ser Val Ala Ile Ser His Gly Arg
                565                 570                 575

Leu Val Val Ala Asp Ser Glu Gly Lys Leu Phe Ser Ala Ala Ile Pro
            580                 585                 590

Lys Gln Gly Asp Gly Asn Glu Leu Lys Met Lys Ala Met Pro Gln His
        595                 600                 605

Ala Leu Asp Glu His Phe Gly His Asp His Gln Ile Ser Gly Phe Phe
    610                 615                 620

His Asp Asp His Gly Gln Leu Asn Ala Leu Val Lys Asn Asn Phe Arg
625                 630                 635                 640

Gln Gln His Ala Cys Pro Leu Gly Asn Asp His Gln Phe His Pro Gly
                645                 650                 655

Trp Asn Leu Thr Asp Ala Leu Val Ile Asp Asn Gln Leu Gly Leu His
            660                 665                 670

His Thr Asn Pro Glu Pro His Glu Ile Leu Asp Met Gly His Leu Gly
        675                 680                 685

Ser Leu Ala Leu Gln Glu Gly Lys Leu His Tyr Phe Asp Gln Leu Thr
    690                 695                 700

Lys Gly Trp Thr Gly Ala Glu Ser Asp Cys Lys Gln Leu Lys Lys Gly
705                 710                 715                 720

Leu Asp Gly Ala Ala Tyr Leu Leu Lys Asp Gly Glu Val Lys Arg Leu
                725                 730                 735

Asn Ile Asn Gln Ser Thr Ser Ser Ile Lys His Gly Thr Glu Asn Val
            740                 745                 750

Phe Ser Leu Pro His Val Arg Asn Lys Pro Glu Pro Gly Asp Ala Leu
        755                 760                 765

Gln Gly Leu Asn Lys Asp Asp Lys Ala Gln Ala Met Ala Val Ile Gly
    770                 775                 780

Val Asn Lys Tyr Leu Ala Leu Thr Glu Lys Gly Asp Ile Arg Ser Phe
785                 790                 795                 800

Gln Ile Lys Pro Gly Thr Gln Gln Leu Glu Arg Pro Ala Gln Thr Leu
                805                 810                 815

Ser Arg Glu Gly Ile Ser Gly Glu Leu Lys Asp Ile His Val Asp His
            820                 825                 830

Lys Gln Asn Leu Tyr Ala Leu Thr His Glu Gly Glu Val Phe His Gln
        835                 840                 845

Pro Arg Glu Ala Trp Gln Asn Gly Ala Glu Ser Ser Ser Trp His Lys
    850                 855                 860

Leu Ala Leu Pro Gln Ser Glu Ser Lys Leu Lys Ser Leu Asp Met Ser
865                 870                 875                 880

His Glu His Lys Pro Ile Ala Thr Phe Glu Asp Gly Ser Gln His Gln
                885                 890                 895
```

```
Leu Lys Ala Gly Gly Trp His Ala Tyr Ala Ala Pro Glu Arg Gly Pro
            900                 905                 910

Leu Ala Val Gly Thr Ser Gly Ser Gln Thr Val Phe Asn Arg Leu Met
        915                 920                 925

Gln Gly Val Lys Gly Lys Val Ile Pro Gly Ser Gly Leu Thr Val Lys
    930                 935                 940

Leu Ser Ala Gln Thr Gly Gly Met Thr Gly Ala Glu Gly Arg Lys Val
945                 950                 955                 960

Ser Ser Lys Phe Ser Glu Arg Ile Arg Ala Tyr Ala Phe Asn Pro Thr
                965                 970                 975

Met Ser Thr Pro Arg Pro Ile Lys Asn Ala Ala Tyr Ala Thr Gln His
            980                 985                 990

Gly Trp Gln Gly Arg Glu Gly Leu Lys Pro Leu Tyr Glu Met Gln Gly
        995                 1000                1005

Ala Leu Ile Lys Gln Leu Asp Ala His Asn Val Arg His Asn Ala Pro
    1010                1015                1020

Gln Pro Asp Leu Gln Ser Lys Leu Glu Thr Leu Asp Leu Gly Glu His
1025                1030                1035                1040

Gly Ala Glu Leu Leu Asn Asp Met Lys Arg Phe Arg Asp Glu Leu Glu
                1045                1050                1055

Gln Ser Ala Thr Arg Ser Val Thr Val Leu Gly Gln His Gln Gly Val
            1060                1065                1070

Leu Lys Ser Asn Gly Glu Ile Asn Ser Glu Phe Lys Pro Ser Pro Gly
        1075                1080                1085

Lys Ala Leu Val Gln Ser Phe Asn Val Asn Arg Ser Gly Gln Asp Leu
    1090                1095                1100

Ser Lys Ser Leu Gln Gln Ala Val His Ala Thr Pro Pro Ser Ala Glu
1105                1110                1115                1120

Ser Lys Leu Gln Ser Met Leu Gly His Phe Val Ser Ala Gly Val Asp
                1125                1130                1135

Met Ser His Gln Lys Gly Glu Ile Pro Leu Gly Arg Gln Arg Asp Pro
            1140                1145                1150

Asn Asp Lys Thr Ala Leu Thr Lys Ser Arg Leu Ile Leu Asp Thr Val
        1155                1160                1165

Thr Ile Gly Glu Leu His Glu Leu Ala Asp Lys Ala Lys Leu Val Ser
    1170                1175                1180

Asp His Lys Pro Asp Ala Asp Gln Ile Lys Gln Leu Arg Gln Gln Phe
1185                1190                1195                1200

Asp Thr Leu Arg Glu Lys Arg Tyr Glu Ser Asn Pro Val Lys His Tyr
                1205                1210                1215

Thr Asp Met Gly Phe Thr His Asn Lys Ala Leu Glu Ala Asn Tyr Asp
            1220                1225                1230

Ala Val Lys Ala Phe Ile Asn Ala Phe Lys Lys Glu His His Gly Val
        1235                1240                1245
```

```
Asn Leu Thr Thr Arg Thr Val Leu Glu Ser Gln Gly Ser Ala Glu Leu
    1250                1255                1260

Ala Lys Lys Leu Lys Asn Thr Leu Leu Ser Leu Asp Ser Gly Glu Ser
1265                1270                1275                1280

Met Ser Phe Ser Arg Ser Tyr Gly Gly Gly Val Ser Thr Val Phe Val
                1285                1290                1295

Pro Thr Leu Ser Lys Lys Val Pro Val Pro Val Ile Pro Gly Ala Gly
            1300                1305                1310

Ile Thr Leu Asp Arg Ala Tyr Asn Leu Ser Phe Ser Arg Thr Ser Gly
        1315                1320                1325

Gly Leu Asn Val Ser Phe Gly Arg Asp Gly Gly Val Ser Gly Asn Ile
    1330                1335                1340

Met Val Ala Thr Gly His Asp Val Met Pro Tyr Met Thr Gly Lys Lys
1345                1350                1355                1360

Thr Ser Ala Gly Asn Ala Ser Asp Trp Leu Ser Ala Lys His Lys Ile
                1365                1370                1375

Ser Pro Asp Leu Arg Ile Gly Ala Ala Val Ser Gly Thr Leu Gln Gly
            1380                1385                1390

Thr Leu Gln Asn Ser Leu Lys Phe Lys Leu Thr Glu Asp Glu Leu Pro
        1395                1400                1405

Gly Phe Ile His Gly Leu Thr His Gly Thr Leu Thr Pro Ala Glu Leu
    1410                1415                1420

Leu Gln Lys Gly Ile Glu His Gln Met Lys Gln Gly Ser Lys Leu Thr
1425                1430                1435                1440

Phe Ser Val Asp Thr Ser Ala Asn Leu Asp Leu Arg Ala Gly Ile Asn
                1445                1450                1455

Leu Asn Glu Asp Gly Ser Lys Pro Asn Gly Val Thr Ala Arg Val Ser
            1460                1465                1470

Ala Gly Leu Ser Ala Ser Ala Asn Leu Ala Ala Gly Ser Arg Glu Arg
        1475                1480                1485

Ser Thr Thr Ser Gly Gln Phe Gly Ser Thr Thr Ser Ala Ser Asn Asn
    1490                1495                1500

Arg Pro Thr Phe Leu Asn Gly Val Gly Ala Gly Ala Asn Leu Thr Ala
1505                1510                1515                1520

Ala Leu Gly Val Ala His Ser Ser Thr His Glu Gly Lys Pro Val Gly
                1525                1530                1535

Ile Phe Pro Ala Phe Thr Ser Thr Asn Val Ser Ala Ala Leu Ala Leu
            1540                1545                1550

Asp Asn Arg Thr Ser Gln Ser Ile Ser Leu Glu Leu Lys Arg Ala Glu
        1555                1560                1565

Pro Val Thr Ser Asn Asp Ile Ser Glu Leu Thr Ser Thr Leu Gly Lys
    1570                1575                1580
```

```
His Phe Lys Asp Ser Ala Thr Thr Lys Met Leu Ala Ala Leu Lys Glu
1585                1590                1595                1600

Leu Asp Asp Ala Lys Pro Ala Glu Gln Leu His Ile Leu Gln Gln His
                1605                1610                1615

Phe Ser Ala Lys Asp Val Val Gly Asp Glu Arg Tyr Glu Ala Val Arg
            1620                1625                1630

Asn Leu Lys Lys Leu Val Ile Arg Gln Gln Ala Ala Asp Ser His Ser
        1635                1640                1645

Met Glu Leu Gly Ser Ala Ser His Ser Thr Thr Tyr Asn Asn Leu Ser
    1650                1655                1660

Arg Ile Asn Asn Asp Gly Ile Val Glu Leu Leu His Lys His Phe Asp
1665                1670                1675                1680

Ala Ala Leu Pro Ala Ser Ser Ala Lys Arg Leu Gly Glu Met Met Asn
                1685                1690                1695

Asn Asp Pro Ala Leu Lys Asp Ile Ile Lys Gln Leu Gln Ser Thr Pro
            1700                1705                1710

Phe Ser Ser Ala Ser Val Ser Met Glu Leu Lys Asp Gly Leu Arg Glu
        1715                1720                1725

Gln Thr Glu Lys Ala Ile Leu Asp Gly Lys Val Gly Arg Glu Glu Val
    1730                1735                1740

Gly Val Leu Phe Gln Asp Arg Asn Asn Leu Arg Val Lys Ser Val Ser
1745                1750                1755                1760

Val Ser Gln Ser Val Ser Lys Ser Glu Gly Phe Asn Thr Pro Ala Leu
                1765                1770                1775

Leu Leu Gly Thr Ser Asn Ser Ala Ala Met Ser Met Glu Arg Asn Ile
            1780                1785                1790

Gly Thr Ile Asn Phe Lys Tyr Gly Gln Asp Gln Asn Thr Pro Arg Arg
        1795                1800                1805

Phe Thr Leu Glu Gly Gly Ile Ala Gln Ala Asn Pro Gln Val Ala Ser
    1810                1815                1820

Ala Leu Thr Asp Leu Lys Lys Glu Gly Leu Glu Met Lys Ser
1825                1830                1835
```

This protein or polypeptide is about 198 kDa and has a pI of 8.98.

The present invention relates to an isolated DNA molecule having a nucleotide sequence of SEQ. ID. No. 9 as follows:

```
ATGACATCGT CACAGCAGCG GGTTGAAAGG TTTTTACAGT ATTTCTCCGC CGGGTGTAAA     60
ACGCCCATAC ATCTGAAAGA CGGGGTGTGC GCCCTGTATA ACGAACAAGA TGAGGAGGCG    120
GCGGTGCTGG AAGTACCGCA ACACAGCGAC AGCCTGTTAC TACACTGCCG AATCATTGAG    180
GCTGACCCAC AAACTTCAAT AACCCTGTAT TCGATGCTAT TACAGCTGAA TTTTGAAATG    240
```

```
GCGGCCATGC GCGGCTGTTG GCTGGCGCTG GATGAACTGC ACAACGTGCG TTTATGTTTT     300

CAGCAGTCGC TGGAGCATCT GGATGAAGCA AGTTTTAGCG ATATCGTTAG CGGCTTCATC     360

GAACATGCGG CAGAAGTGCG TGAGTATATA GCGCAATTAG ACGAGAGTAG CGCGGCATAA     420
```

This is known as the dspF gene. This isolated DNA molecule of the present invention encodes a hypersensitive response elicitor protein or polypeptide having an amino acid sequence of SEQ. ID. No. 10 as follows:

```
Met Thr Ser Ser Gln Gln Arg Val Glu Arg Phe Leu Gln Tyr Phe Ser
1               5                  10                  15

Ala Gly Cys Lys Thr Pro Ile His Leu Lys Asp Gly Val Cys Ala Leu
            20                  25                  30

Tyr Asn Glu Gln Asp Glu Glu Ala Ala Val Leu Glu Val Pro Gln His
        35                  40                  45

Ser Asp Ser Leu Leu Leu His Cys Arg Ile Ile Glu Ala Asp Pro Gln
    50                  55                  60

Thr Ser Ile Thr Leu Tyr Ser Met Leu Leu Gln Leu Asn Phe Glu Met
65                  70                  75                  80

Ala Ala Met Arg Gly Cys Trp Leu Ala Leu Asp Glu Leu His Asn Val
                85                  90                  95

Arg Leu Cys Phe Gln Gln Ser Leu Glu His Leu Asp Glu Ala Ser Phe
            100                 105                 110

Ser Asp Ile Val Ser Gly Phe Ile Glu His Ala Ala Glu Val Arg Glu
        115                 120                 125

Tyr Ile Ala Gln Leu Asp Glu Ser Ser Ala Ala
    130                 135
```

This protein or polypeptide is about 16 kDa and has a pI of 4.45.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas syringae* has an amino acid sequence corresponding to SEQ. ID. No. 11 as follows:

```
Met Gln Ser Leu Ser Leu Asn Ser Ser Ser Leu Gln Thr Pro Ala Met
1               5                  10                  15

Ala Leu Val Leu Val Arg Pro Glu Ala Glu Thr Thr Gly Ser Thr Ser
            20                  25                  30

Ser Lys Ala Leu Gln Glu Val Val Val Lys Leu Ala Glu Glu Leu Met
        35                  40                  45

Arg Asn Gly Gln Leu Asp Asp Ser Ser Pro Leu Gly Lys Leu Leu Ala
    50                  55                  60
```

```
Lys Ser Met Ala Ala Asp Gly Lys Ala Gly Gly Gly Ile Glu Asp Val
65                  70                  75                  80

Ile Ala Ala Leu Asp Lys Leu Ile His Glu Lys Leu Gly Asp Asn Phe
                85                  90                  95

Gly Ala Ser Ala Asp Ser Ala Ser Gly Thr Gly Gln Gln Asp Leu Met
            100                 105                 110

Thr Gln Val Leu Asn Gly Leu Ala Lys Ser Met Leu Asp Asp Leu Leu
        115                 120                 125

Thr Lys Gln Asp Gly Gly Thr Ser Phe Ser Glu Asp Asp Met Pro Met
    130                 135                 140

Leu Asn Lys Ile Ala Gln Phe Met Asp Asp Asn Pro Ala Gln Phe Pro
145                 150                 155                 160

Lys Pro Asp Ser Gly Ser Trp Val Asn Glu Leu Lys Glu Asp Asn Phe
                165                 170                 175

Leu Asp Gly Asp Glu Thr Ala Ala Phe Arg Ser Ala Leu Asp Ile Ile
            180                 185                 190

Gly Gln Gln Leu Gly Asn Gln Gln Ser Asp Ala Gly Ser Leu Ala Gly
        195                 200                 205

Thr Gly Gly Gly Leu Gly Thr Pro Ser Ser Phe Ser Asn Asn Ser Ser
    210                 215                 220

Val Met Gly Asp Pro Leu Ile Asp Ala Asn Thr Gly Pro Gly Asp Ser
225                 230                 235                 240

Gly Asn Thr Arg Gly Glu Ala Gly Gln Leu Ile Gly Glu Leu Ile Asp
                245                 250                 255

Arg Gly Leu Gln Ser Val Leu Ala Gly Gly Gly Leu Gly Thr Pro Val
            260                 265                 270

Asn Thr Pro Gln Thr Gly Thr Ser Ala Asn Gly Gly Gln Ser Ala Gln
        275                 280                 285

Asp Leu Asp Gln Leu Leu Gly Gly Leu Leu Leu Lys Gly Leu Glu Ala
    290                 295                 300

Thr Leu Lys Asp Ala Gly Gln Thr Gly Thr Asp Val Gln Ser Ser Ala
305                 310                 315                 320

Ala Gln Ile Ala Thr Leu Leu Val Ser Thr Leu Leu Gln Gly Thr Arg
                325                 330                 335

Asn Gln Ala Ala Ala
            340
```

This hypersensitive response elicitor polypeptide or protein has a molecular weight of 34-35 kDa. It is rich in glycine (about 13.5%) and lacks cysteine and tyrosine. Further information about the hypersensitive response elicitor derived from *Pseudomonas syringae* is found in He, S. Y., H. C. Huang, and A. Collmer, "*Pseudomonas syringae* pv. *syringae* $Harpin_{Pss}$: a Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266 (1993), which is hereby incorporated by reference. The DNA molecule encoding the hypersensitive response elicitor from *Pseudomonas syringae* has a nucleotide sequence corresponding to SEQ. ID. No. 12 as follows:

```
ATGCAGAGTC TCAGTCTTAA CAGCAGCTCG CTGCAAACCC CGGCAATGGC CCTTGTCCTG      60
GTACGTCCTG AAGCCGAGAC GACTGGCAGT ACGTCGAGCA AGGCGCTTCA GGAAGTTGTC     120
GTGAAGCTGG CCGAGGAACT GATGCGCAAT GGTCAACTCG ACGACAGCTC GCCATTGGGA     180
AAACTGTTGG CCAAGTCGAT GGCCGCAGAT GGCAAGGCGG GCGGCGGTAT TGAGGATGTC     240
ATCGCTGCGC TGGACAAGCT GATCCATGAA AAGCTCGGTG ACAACTTCGG CGCGTCTGCG     300
GACAGCGCCT CGGGTACCGG ACAGCAGGAC CTGATGACTC AGGTGCTCAA TGGCCTGGCC     360
AAGTCGATGC TCGATGATCT TCTGACCAAG CAGGATGGCG GGACAAGCTT CTCCGAAGAC     420
GATATGCCGA TGCTGAACAA GATCGCGCAG TTCATGGATG ACAATCCCGC ACAGTTTCCC     480
AAGCCGGACT CGGGCTCCTG GGTGAACGAA CTCAAGGAAG ACAACTTCCT TGATGGCGAC     540
GAAACGGCTG CGTTCCGTTC GGCACTCGAC ATCATTGGCC AGCAACTGGG TAATCAGCAG     600
AGTGACGCTG GCAGTCTGGC AGGGACGGGT GGAGGTCTGG GCACTCCGAG CAGTTTTTCC     660
AACAACTCGT CCGTGATGGG TGATCCGCTG ATCGACGCCA ATACCGGTCC CGGTGACAGC     720
GGCAATACCC GTGGTGAAGC GGGGCAACTG ATCGGCGAGC TTATCGACCG TGGCCTGCAA     780
TCGGTATTGG CCGGTGGTGG ACTGGGCACA CCCGTAAACA CCCCGCAGAC CGGTACGTCG     840
GCGAATGGCG GACAGTCCGC TCAGGATCTT GATCAGTTGC TGGGCGGCTT GCTGCTCAAG     900
GGCCTGGAGG CAACGCTCAA GGATGCCGGG CAAACAGGCA CCGACGTGCA GTCGAGCGCT     960
GCGCAAATCG CCACCTTGCT GGTCAGTACG CTGCTGCAAG GCACCCGCAA TCAGGCTGCA    1020
GCCTGA                                                               1026
```

Another potentially suitable hypersensitive response elicitor from *Pseudomonas syringae* is disclosed in U.S. Patent Application Serial No. 09/120,817,

which is hereby incorporated by reference. The protein has a nucleotide sequence of SEQ. ID. No. 13 as follows:

```
TCCACTTCGC TGATTTTGAA ATTGGCAGAT TCATAGAAAC GTTCAGGTGT GGAAATCAGG      60
CTGAGTGCGC AGATTTCGTT GATAAGGGTG TGGTACTGGT CATTGTTGGT CATTTCAAGG     120
CCTCTGAGTG CGGTGCGGAG CAATACCAGT CTTCCTGCTG GCGTGTGCAC ACTGAGTCGC     180
AGGCATAGGC ATTTCAGTTC CTTGCGTTGG TTGGGCATAT AAAAAAAGGA ACTTTTAAAA     240
ACAGTGCAAT GAGATGCCGG CAAAACGGGA ACCGGTCGCT GCGCTTTGCC ACTCACTTCG     300
AGCAAGCTCA ACCCCAAACA TCCACATCCC TATCGAACGG ACAGCGATAC GGCCACTTGC     360
TCTGGTAAAC CCTGGAGCTG GCGTCGGTCC AATTGCCCAC TTAGCGAGGT AACGCAGCAT     420
GAGCATCGGC ATCACACCCC GGCCGCAACA GACCACCACG CCACTCGATT TTTCGGCGCT     480
AAGCGGCAAG AGTCCTCAAC CAAACACGTT CGGCGAGCAG AACACTCAGC AAGCGATCGA     540
CCCGAGTGCA CTGTTGTTCG GCAGCGACAC ACAGAAAGAC GTCAACTTCG GCACGCCCGA     600
CAGCACCGTC CAGAATCCGC AGGACGCCAG CAAGCCCAAC GACAGCCAGT CCAACATCGC     660
TAAATTGATC AGTGCATTGA TCATGTCGTT GCTGCAGATG CTCACCAACT CCAATAAAAA     720
GCAGGACACC AATCAGGAAC AGCCTGATAG CCAGGCTCCT TTCCAGAACA ACGGCGGGCT     780
CGGTACACCG TCGGCCGATA GCGGGGGCGG CGGTACACCG GATGCGACAG GTGGCGGCGG     840
CGGTGATACG CCAAGCGCAA CAGGCGGTGG CGGCGGTGAT ACTCCGACCG CAACAGGCGG     900
TGGCGGCAGC GGTGGCGGCG GCACACCCAC TGCAACAGGT GGCGGCAGCG GTGGCACACC     960
CACTGCAACA GGCGGTGGCG AGGGTGGCGT AACACCGCAA ATCACTCCGC AGTTGGCCAA    1020
CCCTAACCGT ACCTCAGGTA CTGGCTCGGT GTCGGACACC GCAGGTTCTA CCGAGCAAGC    1080
CGGCAAGATC AATGTGGTGA AAGACACCAT CAAGGTCGGC GCTGGCGAAG TCTTTGACGG    1140
CCACGGCGCA ACCTTCACTG CCGACAAATC TATGGGTAAC GGAGACCAGG GCGAAAATCA    1200
GAAGCCCATG TTCGAGCTGG CTGAAGGCGC TACGTTGAAG AATGTGAACC TGGGTGAGAA    1260
CGAGGTCGAT GGCATCCACG TGAAAGCCAA AAACGCTCAG GAAGTCACCA TTGACAACGT    1320
GCATGCCCAG AACGTCGGTG AAGACCTGAT TACGGTCAAA GGCGAGGGAG GCGCAGCGGT    1380
CACTAATCTG AACATCAAGA ACAGCAGTGC CAAAGGTGCA GACGACAAGG TTGTCCAGCT    1440
CAACGCCAAC ACTCACTTGA AAATCGACAA CTTCAAGGCC GACGATTTCG GCACGATGGT    1500
TCGCACCAAC GGTGGCAAGC AGTTTGATGA CATGAGCATC GAGCTGAACG GCATCGAAGC    1560
TAACCACGGC AAGTTCGCCC TGGTGAAAAG CGACAGTGAC GATCTGAAGC TGGCAACGGG    1620
CAACATCGCC ATGACCGACG TCAAACACGC CTACGATAAA ACCCAGGCAT CGACCCAACA    1680
CACCGAGCTT TGAATCCAGA CAAGTAGCTT GAAAAAAGGG GGTGGACTC                1729
```

This DNA molecule is known as the dspE gene for *Pseudomonas syringae*. This isolated DNA molecule of the present invention encodes a protein or polypeptide which elicits a plant pathogen's hypersensitive response having an amino acid sequence of SEQ. ID. No. 14 as follows:

```
Met Ser Ile Gly Ile Thr Pro Arg Pro Gln Gln Thr Thr Thr Pro Leu
1               5                  10                  15

Asp Phe Ser Ala Leu Ser Gly Lys Ser Pro Gln Pro Asn Thr Phe Gly
            20                  25                  30

Glu Gln Asn Thr Gln Gln Ala Ile Asp Pro Ser Ala Leu Leu Phe Gly
        35                  40                  45

Ser Asp Thr Gln Lys Asp Val Asn Phe Gly Thr Pro Asp Ser Thr Val
    50                  55                  60

Gln Asn Pro Gln Asp Ala Ser Lys Pro Asn Asp Ser Gln Ser Asn Ile
65                  70                  75                  80

Ala Lys Leu Ile Ser Ala Leu Ile Met Ser Leu Leu Gln Met Leu Thr
                85                  90                  95

Asn Ser Asn Lys Lys Gln Asp Thr Asn Gln Glu Gln Pro Asp Ser Gln
            100                 105                 110

Ala Pro Phe Gln Asn Asn Gly Gly Leu Gly Thr Pro Ser Ala Asp Ser
        115                 120                 125

Gly Gly Gly Gly Thr Pro Asp Ala Thr Gly Gly Gly Gly Gly Asp Thr
    130                 135                 140

Pro Ser Ala Thr Gly Gly Gly Gly Gly Asp Thr Pro Thr Ala Thr Gly
145                 150                 155                 160

Gly Gly Gly Ser Gly Gly Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly
                165                 170                 175

Ser Gly Gly Thr Pro Thr Ala Thr Gly Gly Gly Glu Gly Gly Val Thr
            180                 185                 190

Pro Gln Ile Thr Pro Gln Leu Ala Asn Pro Asn Arg Thr Ser Gly Thr
        195                 200                 205

Gly Ser Val Ser Asp Thr Ala Gly Ser Thr Glu Gln Ala Gly Lys Ile
    210                 215                 220

Asn Val Val Lys Asp Thr Ile Lys Val Gly Ala Gly Glu Val Phe Asp
225                 230                 235                 240

Gly His Gly Ala Thr Phe Thr Ala Asp Lys Ser Met Gly Asn Gly Asp
                245                 250                 255

Gln Gly Glu Asn Gln Lys Pro Met Phe Glu Leu Ala Glu Gly Ala Thr
            260                 265                 270
```

```
Leu Lys Asn Val Asn Leu Gly Glu Asn Glu Val Asp Gly Ile His Val
        275                 280                 285

Lys Ala Lys Asn Ala Gln Glu Val Thr Ile Asp Asn Val His Ala Gln
    290                 295                 300

Asn Val Gly Glu Asp Leu Ile Thr Val Lys Gly Glu Gly Gly Ala Ala
305                 310                 315                 320

Val Thr Asn Leu Asn Ile Lys Asn Ser Ser Ala Lys Gly Ala Asp Asp
                325                 330                 335

Lys Val Val Gln Leu Asn Ala Asn Thr His Leu Lys Ile Asp Asn Phe
            340                 345                 350

Lys Ala Asp Asp Phe Gly Thr Met Val Arg Thr Asn Gly Gly Lys Gln
        355                 360                 365

Phe Asp Asp Met Ser Ile Glu Leu Asn Gly Ile Glu Ala Asn His Gly
    370                 375                 380

Lys Phe Ala Leu Val Lys Ser Asp Ser Asp Asp Leu Lys Leu Ala Thr
385                 390                 395                 400

Gly Asn Ile Ala Met Thr Asp Val Lys His Ala Tyr Asp Lys Thr Gln
                405                 410                 415

Ala Ser Thr Gln His Thr Glu Leu
            420
```

This protein or polypeptide is about 42.9 kDa.

This hypersensitive response elicitor from *Pseudomonas syringae* has 1 hypersensitive response eliciting domain. This domain extends, within SEQ. ID. No. 14, from amino acid 45 to amino acid 102, particularly from amino acid 58 to amino acid 92. The acidic unit in the first domain extends, within SEQ. ID. No. 14, from amino acid 45 to amino acid 79, particularly from amino acid 58 to amino acid 79. The alpha-helix in the first domain extends, within SEQ. ID. No. 14, from amino acid 79 to amino acid 102, particularly from amino acid 79 to amino acid 92.

The hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* has an amino acid sequence corresponding to SEQ. ID. No. 15 as follows:

```
Met Ser Val Gly Asn Ile Gln Ser Pro Ser Asn Leu Pro Gly Leu Gln
1               5                   10                  15

Asn Leu Asn Leu Asn Thr Asn Thr Asn Ser Gln Gln Ser Gly Gln Ser
            20                  25                  30
```

```
Val Gln Asp Leu Ile Lys Gln Val Glu Lys Asp Ile Leu Asn Ile Ile
        35                  40                  45

Ala Ala Leu Val Gln Lys Ala Ala Gln Ser Ala Gly Gly Asn Thr Gly
    50                  55                  60

Asn Thr Gly Asn Ala Pro Ala Lys Asp Gly Asn Ala Asn Ala Gly Ala
65                  70                  75                  80

Asn Asp Pro Ser Lys Asn Asp Pro Ser Lys Ser Gln Ala Pro Gln Ser
                85                  90                  95

Ala Asn Lys Thr Gly Asn Val Asp Asp Ala Asn Asn Gln Asp Pro Met
            100                 105                 110

Gln Ala Leu Met Gln Leu Leu Glu Asp Leu Val Lys Leu Leu Lys Ala
        115                 120                 125

Ala Leu His Met Gln Gln Pro Gly Gly Asn Asp Lys Gly Asn Gly Val
    130                 135                 140

Gly Gly Ala Asn Gly Ala Lys Gly Ala Gly Gly Gln Gly Gly Leu Ala
145                 150                 155                 160

Glu Ala Leu Gln Glu Ile Glu Gln Ile Leu Ala Gln Leu Gly Gly Gly
                165                 170                 175

Gly Ala Gly Ala Gly Gly Ala Gly Gly Gly Val Gly Gly Ala Gly Gly
            180                 185                 190

Ala Asp Gly Gly Ser Gly Ala Gly Gly Ala Gly Gly Ala Asn Gly Ala
        195                 200                 205

Asp Gly Gly Asn Gly Val Asn Gly Asn Gln Ala Asn Gly Pro Gln Asn
    210                 215                 220

Ala Gly Asp Val Asn Gly Ala Asn Gly Ala Asp Asp Gly Ser Glu Asp
225                 230                 235                 240

Gln Gly Gly Leu Thr Gly Val Leu Gln Lys Leu Met Lys Ile Leu Asn
                245                 250                 255

Ala Leu Val Gln Met Met Gln Gln Gly Gly Leu Gly Gly Gly Asn Gln
            260                 265                 270

Ala Gln Gly Gly Ser Lys Gly Ala Gly Asn Ala Ser Pro Ala Ser Gly
        275                 280                 285

Ala Asn Pro Gly Ala Asn Gln Pro Gly Ser Ala Asp Asp Gln Ser Ser
    290                 295                 300

Gly Gln Asn Asn Leu Gln Ser Gln Ile Met Asp Val Val Lys Glu Val
305                 310                 315                 320

Val Gln Ile Leu Gln Gln Met Leu Ala Ala Gln Asn Gly Gly Ser Gln
                325                 330                 335
```

Gln Ser Thr Ser Thr Gln Pro Met
340

It is encoded by a DNA molecule having a nucleotide sequence corresponding SEQ. ID. No. 16 as follows:

```
ATGTCAGTCG GAAACATCCA GAGCCCGTCG AACCTCCCGG GTCTGCAGAA CCTGAACCTC      60
AACACCAACA CCAACAGCCA GCAATCGGGC CAGTCCGTGC AAGACCTGAT CAAGCAGGTC     120
GAGAAGGACA TCCTCAACAT CATCGCAGCC CTCGTGCAGA AGGCCGCACA GTCGGCGGGC     180
GGCAACACCG GTAACACCGG CAACGCGCCG GCGAAGGACG GCAATGCCAA CGCGGGCGCC     240
AACGACCCGA GCAAGAACGA CCCGAGCAAG AGCCAGGCTC CGCAGTCGGC CAACAAGACC     300
GGCAACGTCG ACGACGCCAA CAACCAGGAT CCGATGCAAG CGCTGATGCA GCTGCTGGAA     360
GACCTGGTGA AGCTGCTGAA GGCGGCCCTG CACATGCAGC AGCCCGGCGG CAATGACAAG     420
GGCAACGGCG TGGGCGGTGC CAACGGCGCC AAGGGTGCCG GCGGCCAGGG CGGCCTGGCC     480
GAAGCGCTGC AGGAGATCGA GCAGATCCTC GCCCAGCTCG GCGGCGGCGG TGCTGGCGCC     540
GGCGGCGCGG GTGGCGGTGT CGGCGGTGCT GGTGGCGCGG ATGGCGGCTC CGGTGCGGGT     600
GGCGCAGGCG GTGCGAACGG CGCCGACGGC GGCAATGGCG TGAACGGCAA CCAGGCGAAC     660
GGCCCGCAGA ACGCAGGCGA TGTCAACGGT GCCAACGGCG CGGATGACGG CAGCGAAGAC     720
CAGGGCGGCC TCACCGGCGT GCTGCAAAAG CTGATGAAGA TCCTGAACGC GCTGGTGCAG     780
ATGATGCAGC AAGGCGGCCT CGGCGGCGGC AACCAGGCGC AGGGCGGCTC GAAGGGTGCC     840
GGCAACGCCT CGCCGGCTTC CGGCGCGAAC CCGGGCGCGA ACCAGCCCGG TTCGGCGGAT     900
GATCAATCGT CCGGCCAGAA CAATCTGCAA TCCCAGATCA TGGATGTGGT GAAGGAGGTC     960
GTCCAGATCC TGCAGCAGAT GCTGGCGGCG CAGAACGGCG GCAGCCAGCA GTCCACCTCG    1020
ACGCAGCCGA TGTAA                                                     1035
```

Further information regarding the hypersensitive response elicitor polypeptide or protein derived from *Pseudomonas solanacearum* is set forth in Arlat, M., F. Van Gijsegem, J. C. Huet, J. C. Pemollet, and C. A. Boucher, "PopA1, a Protein which Induces a Hypersensitive-like Response in Specific Petunia Genotypes, is Secreted via the Hrp Pathway of *Pseudomonas solanacearum*," EMBO J. 13:543-533 (1994), which is hereby incorporated by reference.

The hypersensitive response elicitor from *Pseudomonas solanacearum* has 2 hypersensitive response eliciting domains. The first domain extends, within SEQ. ID.

No. 15, from amino acid 85 to amino acid 131, particularly from amino acid 95 to amino acid 123. The acidic unit in the first domain extends, within SEQ. ID. No. 15, from amino acid 85 to amino acid 111, particularly from amino acid 95 to amino acid 123. The alpha-helix in the first domain extends, within SEQ. ID. No. 15, from amino acid 85 to amino acid 111, particularly from amino acid 95 to amino acid 111. The second domain extends, within SEQ. ID. No. 15, from amino acid 195 to amino acid 264, particularly from amino acid 229 to amino acid 258. The acidic unit in the second domain extends, within SEQ. ID. No. 15, from amino acid 195 to amino acid 246, particularly from amino acid 229 to amino acid 264. The alpha-helix in the second domain extends, within SEQ. ID. No. 15, from amino acid 246 to amino acid 264, particularly from amino acid 246 to amino acid 258.

The N-terminus of the hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris* has an amino acid sequence corresponding to SEQ. ID. No. 17 as follows:

```
Met Asp Gly Ile Gly Asn His Phe Ser Asn
 1               5                  10
```

The hypersensitive response elicitor polypeptide or protein from *Xanthomonas campestris pv. pelargonii* is heat stable, protease sensitive, and has a molecular weight of 20 kDa. It includes an amino acid sequence corresponding to SEQ. ID. No. 18 as follows:

```
Ser Ser Gln Gln Ser Pro Ser Ala Gly Ser Glu Gln Gln Leu Asp Gln
1               5                  10                  15

Leu Leu Ala Met
            20
```

Isolation of *Erwinia carotovora* hypersensitive response elictor protein or polypeptide is described in Cui et al., "The RsmA Mutants of *Erwinia carotovora* subsp. *carotovora* Strain Ecc71 Overexpress *hrp* N_{Ecc} and Elicit a Hypersensitive Reaction-like Response in Tobacco Leaves," MPMI, 9(7):565-73 (1996), which is hereby incorporated by reference. The hypersensitive response elicitor protein or polypeptide of *Erwinia stewartii* is set forth in Ahmad et al., "Harpin is Not

Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," 8th Int'l. Cong. Molec. Plant-Microbe Interact., July 14-19, 1996 and Ahmad, et al., "Harpin is Not Necessary for the Pathogenicity of *Erwinia stewartii* on Maize," Ann. Mtg. Am. Phytopath. Soc., July 27-31, 1996, which are hereby incorporated by reference.

Hypersensitive response elicitor proteins or polypeptides from *Phytophthora parasitica, Phytophthora cryptogea, Phytophthora cinnamoni, Phytophthora capsici, Phytophthora megasperma,* and *Phytophora citrophthora* are described in Kaman, et al., "Extracellular Protein Elicitors from Phytophthora: Most Specificity and Induction of Resistance to Bacterial and Fungal Phytopathogens," Molec. Plant-Microbe Interact., 6(1):15-25 (1993), Ricci et al., "Structure and Activity of Proteins from Pathogenic Fungi Phytophthora Eliciting Necrosis and Acquired Resistance in Tobacco," Eur. J. Biochem., 183:555-63 (1989), Ricci et al., "Differential Production of Parasiticein, and Elicitor of Necrosis and Resistance in Tobacco, by Isolates of Phytophthora parasitica," Plant Path. 41:298-307 (1992), Baillreul et al, "A New Elicitor of the Hypersensitive Response in Tobacco: A Fungal Glycoprotein Elicits Cell Death, Expression of Defence Genes, Production of Salicylic Acid, and Induction of Systemic Acquired Resistance," Plant J., 8(4):551-60 (1995), and Bonnet et al., "Acquired Resistance Triggered by Elicitors in Tobacco and Other Plants," Eur. J. Plant Path., 102:181-92 (1996), which are hereby incorporated by reference.

Another hypersensitive response elicitor in accordance with the present invention is from *Clavibacter michiganensis* subsp. s*epedonicus* which is fully described in U.S. Patent Application Serial No. 09/136,625, which is hereby incorporated by reference.

The above elicitors are exemplary. Other elicitors can be identified by growing fungi or bacteria that elicit a hypersensitive response under conditions which genes encoding an elicitor are expressed. Cell-free preparations from culture supernatants can be tested for elicitor activity (i.e. local necrosis) by using them to infiltrate appropriate plant tissues.

Fragments of the above hypersensitive response elicitor polypeptides or proteins as well as fragments of full length elicitors from other pathogens are encompassed by the method of the present invention.

Suitable fragments can be produced by several means. In the first, subclones of the gene encoding a known elicitor protein are produced by conventional molecular genetic manipulation by subcloning gene fragments. The subclones then are expressed *in vitro* or *in vivo* in bacterial cells to yield a smaller protein or peptide that can be tested for elicitor activity according to the procedure described below.

As an alternative, fragments of an elicitor protein can be produced by digestion of a full-length elicitor protein with proteolytic enzymes like chymotrypsin or *Staphylococcus* proteinase A, or trypsin. Different proteolytic enzymes are likely to cleave elicitor proteins at different sites based on the amino acid sequence of the elicitor protein. Some of the fragments that result from proteolysis may be active elicitors of resistance.

In another approach, based on knowledge of the primary structure of the protein, fragments of the elicitor protein gene may be synthesized by using the PCR technique together with specific sets of primers chosen to represent particular portions of the protein. These then would be cloned into an appropriate vector for expression of a truncated peptide or protein.

Chemical synthesis can also be used to make suitable fragments. Such a synthesis is carried out using known amino acid sequences for the elicitor being produced. Alternatively, subjecting a full length elicitor to high temperatures and pressures will produce fragments. These fragments can then be separated by conventional procedures (e.g., chromatography, SDS-PAGE).

An example of suitable fragments of a hypersensitive response elicitor which do elicit a hypersensitive response are *Erwinia amylovora* fragments including a C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, an N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3, or an internal fragment of the amino acid sequence of SEQ. ID. No. 3. The C-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span amino acids 105 and 403 of SEQ. ID. No. 3. The N-terminal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of SEQ. ID. No. 3: 1 and 98, 1 and 104, 1 and 122, 1 and 168, 1 and 218, 1 and 266, 1 and 342, 1 and 321, and 1 and 372. The internal fragment of the amino acid sequence of SEQ. ID. No. 3 can span the following amino acids of

SEQ. ID. No. 3: 76 and 209, 105 and 209, 99 and 209, 137 and 204, 137 and 200, 109 and 204, 109 and 200, 137 and 180, and 105 and 180.

Suitable DNA molecules are those that hybridize to the DNA molecule comprising a nucleotide sequence of SEQ. ID. Nos. 2, 4, 5, 7, 9, 12, 13, and 16 under stringent conditions. An example of suitable high stringency conditions is when hybridization is carried out at 65°C for 20 hours in a medium containing 1M NaCl, 50 mM Tris-HCl, pH 7.4, 10 mM EDTA, 0.1% sodium dodecyl sulfate, 0.2% ficoll, 0.2% polyvinylpyrrolidone, 0.2% bovine serum albumin, 50 μm g/ml *E. coli* DNA. Suitable stringency conditions also include hybridization in a hybridization buffer comprising 0.9M sodium citrate ("SSC") buffer at a temperature of 37°C where hybridized nucleic acids remain bound when subject to washing the SSC buffer at a temperature of 37°C; and preferably in a hybridization buffer comprising 20% formamide in 0.9M SSC buffer at a temperature of 42°C where hybridized nucleic acids remain bound when subject to washing at 42°C with 0.2x SSC buffer at 42°C.

Variants may be made by, for example, the deletion or addition of amino acids that have minimal influence on the properties, secondary structure and hydropathic nature of the polypeptide. For example, a polypeptide may be conjugated to a signal (or leader) sequence at the N-terminal end of the protein which co-translationally or post-translationally directs transfer of the protein. The polypeptide may also be conjugated to a linker or other sequence for ease of synthesis, purification, or identification of the polypeptide.

A particularly advantageous aspect of the present invention involves utilizing a protein having a pair or more, particularly 3 or more, coupled domains. These domains can be from different source organisms. When a DNA molecule encoding such a protein is prepared, it can be advantageously used to make transgenic plants. The use of a gene encoding such domains, as opposed to a gene encoding a full length hypersensitive response elicitor, has a number of benefits. Firstly, such a gene is easier to synthesize. More significantly, the use of a plurality of domains together from different source organisms can impart their combined benefits to a transgenic plant.

The DNA molecule encoding the hypersensitive response elicitor polypeptide or protein can be incorporated in cells using conventional recombinant

DNA technology. Generally, this involves inserting the DNA molecule into an expression system to which the DNA molecule is heterologous (i.e. not normally present). The heterologous DNA molecule is inserted into the expression system or vector in proper sense orientation and correct reading frame. The vector contains the necessary elements for the transcription and translation of the inserted protein-coding sequences.

U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including procaryotic organisms and eucaryotic cells grown in tissue culture.

Recombinant genes may also be introduced into viruses, such as vaccina virus. Recombinant viruses can be generated by transfection of plasmids into cells infected with virus.

Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gt11, gt WES.tB, Charon 4, and plasmid vectors such as pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKC101, SV 40, pBluescript II SK +/- or KS +/- (see "Stratagene Cloning Systems" Catalog (1993) from Stratagene, La Jolla, Calif, which is hereby incorporated by reference), pQE, pIH821, pGEX, pET series (see F.W. Studier et. al., "Use of T7 RNA Polymerase to Direct Expression of Cloned Genes," Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation. The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Laboratory, Cold Springs Harbor, New York (1989), which is hereby incorporated by reference.

A variety of host-vector systems may be utilized to express the protein-encoding sequence(s). Primarily, the vector system must be compatible with the host cell used. Host-vector systems include but are not limited to the following: bacteria

transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA; microorganisms such as yeast containing yeast vectors; mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, etc.); insect cell systems infected with virus (e.g., baculovirus); and plant cells infected by bacteria. The expression elements of these vectors vary in their strength and specificities. Depending upon the host-vector system utilized, any one of a number of suitable transcription and translation elements can be used.

Different genetic signals and processing events control many levels of gene expression (e.g., DNA transcription and messenger RNA (mRNA) translation).

Transcription of DNA is dependent upon the presence of a promotor which is a DNA sequence that directs the binding of RNA polymerase and thereby promotes mRNA synthesis. The DNA sequences of eucaryotic promotors differ from those of procaryotic promotors. Furthermore, eucaryotic promotors and accompanying genetic signals may not be recognized in or may not function in a procaryotic system, and, further, procaryotic promotors are not recognized and do not function in eucaryotic cells.

Similarly, translation of mRNA in procaryotes depends upon the presence of the proper procaryotic signals which differ from those of eucaryotes. Efficient translation of mRNA in procaryotes requires a ribosome binding site called the Shine-Dalgarno ("SD") sequence on the mRNA. This sequence is a short nucleotide sequence of mRNA that is located before the start codon, usually AUG, which encodes the amino-terminal methionine of the protein. The SD sequences are complementary to the 3'-end of the 16S rRNA (ribosomal RNA) and probably promote binding of mRNA to ribosomes by duplexing with the rRNA to allow correct positioning of the ribosome. For a review on maximizing gene expression, see Roberts and Lauer, Methods in Enzymology, 68:473 (1979), which is hereby incorporated by reference.

Promotors vary in their "strength" (i.e. their ability to promote transcription). For the purposes of expressing a cloned gene, it is desirable to use strong promotors in order to obtain a high level of transcription and, hence, expression of the gene. Depending upon the host cell system utilized, any one of a number of suitable promotors may be used. For instance, when cloning in *E. coli*, its

bacteriophages, or plasmids, promotors such as the T7 phage promoter, *lac* promotor, *trp* promotor, *rec*A promotor, ribosomal RNA promotor, the P_R and P_L promotors of coliphage lambda and others, including but not limited, to *lac*UV5, *omp*F, *bla*, *lpp*, and the like, may be used to direct high levels of transcription of adjacent DNA segments. Additionally, a hybrid *trp-lac*UV5 (*tac*) promotor or other *E. coli* promotors produced by recombinant DNA or other synthetic DNA techniques may be used to provide for transcription of the inserted gene.

Bacterial host cell strains and expression vectors may be chosen which inhibit the action of the promotor unless specifically induced. In certain operations, the addition of specific inducers is necessary for efficient transcription of the inserted DNA. For example, the *lac* operon is induced by the addition of lactose or IPTG (isopropylthio-beta-D-galactoside). A variety of other operons, such as *trp*, *pro*, etc., are under different controls.

Specific initiation signals are also required for efficient gene transcription and translation in procaryotic cells. These transcription and translation initiation signals may vary in "strength" as measured by the quantity of gene specific messenger RNA and protein synthesized, respectively. The DNA expression vector, which contains a promotor, may also contain any combination of various "strong" transcription and/or translation initiation signals. For instance, efficient translation in *E. coli* requires an SD sequence about 7-9 bases 5' to the initiation codon ("ATG") to provide a ribosome binding site. Thus, any SD-ATG combination that can be utilized by host cell ribosomes may be employed. Such combinations include but are not limited to the SD-ATG combination from the *cro* gene or the *N* gene of coliphage lambda, or from the *E. coli* tryptophan E, D, C, B or A genes. Additionally, any SD-ATG combination produced by recombinant DNA or other techniques involving incorporation of synthetic nucleotides may be used.

Once the isolated DNA molecule encoding the hypersensitive response elicitor polypeptide or protein has been cloned into an expression system, it is ready to be incorporated into a host cell. Such incorporation can be carried out by the various forms of transformation noted above, depending upon the vector/host cell system. Suitable host cells include, but are not limited to, plant cells as well as

prokaryotic and eukaryotic cells, such as bacteria, virus, yeast, mammalian, insect cells, and the like.

The present invention further relates to methods of imparting disease resistance to plants, enhancing plant growth, effecting insect control and/or imparting stress resistance to plants. These methods involve applying a hypersensitive response elicitor polypeptide or protein to all or part of a plant or a plant seed under conditions where the polypeptide or protein contacts all or part of the cells of the plant or plant seed. Alternatively, the hypersensitive response elicitor protein or polypeptide can be applied to plants such that seeds recovered from such plants themselves are able to impart disease resistance in plants, to enhance plant growth, to effect insect control, and/or to impart stress resistance.

As an alternative to applying a hypersensitive response elicitor polypeptide or protein to plants or plant seeds in order to impart disease resistance in plants, to effect plant growth, to control insects, and/or to impart stress resistance to the plants or plants grown from the seeds, transgenic plants or plant seeds can be utilized. When utilizing transgenic plants, this involves providing a transgenic plant transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein and growing the plant under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance. Alternatively, a transgenic plant seed transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein can be provided and planted in soil. A plant is then propagated from the planted seed under conditions effective to permit that DNA molecule to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance.

The method of the present invention can be utilized to treat a wide variety of plants or their seeds to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Suitable plants include dicots and monocots. More particularly, useful crop plants can include: alfalfa, rice, wheat, barley, rye, cotton, sunflower, peanut, corn, potato, sweet potato, bean, pea, chicory, lettuce, endive, cabbage, brussel sprout, beet, parsnip, turnip, cauliflower, broccoli, turnip, radish, spinach, onion, garlic, eggplant, pepper, celery, carrot, squash,

pumpkin, zucchini, cucumber, apple, pear, melon, citrus, strawberry, grape, raspberry, pineapple, soybean, tobacco, tomato, sorghum, and sugarcane. Examples of suitable ornamental plants are: *Arabidopsis thaliana*, *Saintpaulia*, petunia, pelargonium, poinsettia, chrysanthemum, carnation, and zinnia.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention in imparting disease resistance, absolute immunity against infection may not be conferred, but the severity of the disease is reduced and symptom development is delayed. Lesion number, lesion size, and extent of sporulation of fungal pathogens are all decreased. This method of imparting disease resistance has the potential for treating previously untreatable diseases, treating diseases systemically which might not be treated separately due to cost, and avoiding the use of infectious agents or environmentally harmful materials.

The method of imparting pathogen resistance to plants in accordance with the present invention is useful in imparting resistance to a wide variety of pathogens including viruses, bacteria, and fungi. Resistance, *inter alia*, to the following viruses can be achieved by the method of the present invention: *Tobacco mosaic virus* and *Tomato mosaic virus*. Resistance, *inter alia*, to the following bacteria can also be imparted to plants in accordance with present invention: *Pseudomonas solancearum*, *Pseudomonas syringae* pv. *tabaci*, and *Xanthamonas campestris* pv. *pelargonii*. Plants can be made resistant, *inter alia*, to the following fungi by use of the method of the present invention: *Fusarium oxysporum* and *Phytophthora infestans*.

With regard to the use of the hypersensitive response elicitor protein or polypeptide of the present invention to enhance plant growth, various forms of plant growth enhancement or promotion can be achieved. This can occur as early as when plant growth begins from seeds or later in the life of a plant. For example, plant growth according to the present invention encompasses greater yield, increased quantity of seeds produced, increased percentage of seeds germinated, increased plant size, greater biomass, more and bigger fruit, earlier fruit coloration, and earlier fruit and plant maturation. As a result, the present invention provides significant economic benefit to growers. For example, early germination and early maturation permit crops to be grown in areas where short growing seasons would otherwise preclude their

growth in that locale. Increased percentage of seed germination results in improved crop stands and more efficient seed use. Greater yield, increased size, and enhanced biomass production allow greater revenue generation from a given plot of land.

Another aspect of the present invention is directed to effecting any form of insect control for plants. For example, insect control according to the present invention encompasses preventing insects from contacting plants to which the hypersensitive response elicitor has been applied, preventing direct insect damage to plants by feeding injury, causing insects to depart from such plants, killing insects proximate to such plants, interfering with insect larval feeding on such plants, preventing insects from colonizing host plants, preventing colonizing insects from releasing phytotoxins, etc. The present invention also prevents subsequent disease damage to plants resulting from insect infection.

The present invention is effective against a wide variety of insects. European corn borer is a major pest of corn (dent and sweet corn) but also feeds on over 200 plant species including green, wax, and lima beans and edible soybeans, peppers, potato, and tomato plus many weed species. Additional insect larval feeding pests which damage a wide variety of vegetable crops include the following: beet armyworm, cabbage looper, corn ear worm, fall armyworm, diamondback moth, cabbage root maggot, onion maggot, seed corn maggot, pickleworm (melonworm), pepper maggot, and tomato pinworm. Collectively, this group of insect pests represents the most economically important group of pests for vegetable production worldwide.

Another aspect of the present invention is directed to imparting stress resistance to plants. Stress encompasses any environmental factor having an adverse effect on plant physiology and development. Examples of such environmental stress include climate-related stress (e.g., drought, water, frost, cold temperature, high temperature, excessive light, and insufficient light), air polllution stress (e.g., carbon dioxide, carbon monoxide, sulfur dioxide, NO_x, hydrocarbons, ozone, ultraviolet radiation, acidic rain), chemical (e.g., insecticides, fungicides, herbicides, heavy metals), and nutritional stress (e.g., fertilizer, micronutrients, macronutrients). Use of hypersensitive response elicitors in accordance with the present invention impart resistance to plants against such forms of environmental stress.

The method of the present invention involving application of the hypersensitive response elicitor polypeptide or protein can be carried out through a variety of procedures when all or part of the plant is treated, including leaves, stems, roots, propagules (e.g., cuttings), etc. This may (but need not) involve infiltration of the hypersensitive response elicitor polypeptide or protein into the plant. Suitable application methods include high or low pressure spraying, injection, and leaf abrasion proximate to when elicitor application takes place. When treating plant seeds, in accordance with the application embodiment of the present invention, the hypersensitive response elicitor protein or polypeptide can be applied by low or high pressure spraying, coating, immersion, or injection. Other suitable application procedures can be envisioned by those skilled in the art provided they are able to effect contact of the hypersensitive response elicitor polypeptide or protein with cells of the plant or plant seed. Once treated with the hypersensitive response elicitor of the present invention, the seeds can be planted in natural or artificial soil and cultivated using conventional procedures to produce plants. After plants have been propagated from seeds treated in accordance with the present invention, the plants may be treated with one or more applications of the hypersensitive response elicitor protein or polypeptide to impart disease resistance to plants, to enhance plant growth, to control insects on the plants, and/or impart stress resistance.

The hypersensitive response elicitor polypeptide or protein can be applied to plants or plant seeds in accordance with the present invention alone or in a mixture with other materials. Alternatively, the hypersensitive response elicitor polypeptide or protein can be applied separately to plants with other materials being applied at different times.

A composition suitable for treating plants or plant seeds in accordance with the application embodiment of the present invention contains a hypersensitive response elicitor polypeptide or protein in a carrier. Suitable carriers include water, aqueous solutions, slurries, or dry powders. In this embodiment, the composition contains greater than 500 nM hypersensitive response elicitor polypeptide or protein.

Although not required, this composition may contain additional additives including fertilizer, insecticide, fungicide, nematacide, and mixtures thereof.

Suitable fertilizers include $(NH_4)_2NO_3$. An example of a suitable insecticide is Malathion. Useful fungicides include Captan.

Other suitable additives include buffering agents, wetting agents, coating agents, and abrading agents. These materials can be used to facilitate the process of the present invention. In addition, the hypersensitive response elicitor polypeptide or protein can be applied to plant seeds with other conventional seed formulation and treatment materials, including clays and polysaccharides.

In the alternative embodiment of the present invention involving the use of transgenic plants and transgenic seeds, a hypersensitive response elicitor polypeptide or protein need not be applied topically to the plants or seeds. Instead, transgenic plants transformed with a DNA molecule encoding a hypersensitive response elicitor polypeptide or protein are produced according to procedures well known in the art.

The vector described above can be microinjected directly into plant cells by use of micropipettes to transfer mechanically the recombinant DNA. Crossway, Mol. Gen. Genetics, 202:179-85 (1985), which is hereby incorporated by reference. The genetic material may also be transferred into the plant cell using polyethylene glycol. Krens, et al., Nature, 296:72-74 (1982), which is hereby incorporated by reference.

Another approach to transforming plant cells with a gene which imparts resistance to pathogens is particle bombardment (also known as biolistic transformation) of the host cell. This can be accomplished in one of several ways. The first involves propelling inert or biologically active particles at cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792, all to Sanford et al., which are hereby incorporated by reference. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and to be incorporated within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the heterologous DNA. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g.,

dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells.

Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes or other fusible lipid-surfaced bodies. Fraley, et al., Proc. Natl. Acad. Sci. USA, 79:1859-63 (1982), which is hereby incorporated by reference.

The DNA molecule may also be introduced into the plant cells by electroporation. Fromm et al., Proc. Natl. Acad. Sci. USA, 82:5824 (1985), which is hereby incorporated by reference. In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate.

Another method of introducing the DNA molecule into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *A. rhizogenes* previously transformed with the gene. Under appropriate conditions known in the art, the transformed plant cells are grown to form shoots or roots, and develop further into plants. Generally, this procedure involves inoculating the plant tissue with a suspension of bacteria and incubating the tissue for 48 to 72 hours on regeneration medium without antibiotics at 25-28°C.

Agrobacterium is a representative genus of the gram-negative family Rhizobiaceae. Its species are responsible for crown gall (*A. tumefaciens*) and hairy root disease (*A. rhizogenes*). The plant cells in crown gall tumors and hairy roots are induced to produce amino acid derivatives known as opines, which are catabolized only by the bacteria. The bacterial genes responsible for expression of opines are a convenient source of control elements for chimeric expression cassettes. In addition, assaying for the presence of opines can be used to identify transformed tissue.

Heterologous genetic sequences can be introduced into appropriate plant cells, by means of the Ti plasmid of *A. tumefaciens* or the Ri plasmid of *A. rhizogenes*. The Ti or Ri plasmid is transmitted to plant cells on infection by Agrobacterium and is stably integrated into the plant genome. J. Schell, Science, 237:1176-83 (1987), which is hereby incorporated by reference.

After transformation, the transformed plant cells must be regenerated.

Plant regeneration from cultured protoplasts is described in Evans et al., Handbook of Plant Cell Cultures, Vol. 1: (MacMillan Publishing Co., New York, 1983); and Vasil I.R. (ed.), Cell Culture and Somatic Cell Genetics of Plants, Acad. Press, Orlando, Vol. I, 1984, and Vol. III (1986), which are hereby incorporated by reference.

It is known that practically all plants can be regenerated from cultured cells or tissues, including but not limited to, all major species of sugarcane, sugar beets, cotton, fruit trees, and legumes.

Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing transformed explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted. Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. It is also advantageous to add glutamic acid and proline to the medium, especially for such species as corn and alfalfa. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

After the expression cassette is stably incorporated in transgenic plants, it can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed.

Once transgenic plants of this type are produced, the plants themselves can be cultivated in accordance with conventional procedure with the presence of the gene encoding the hypersensitive response elicitor resulting in disease resistance, enhanced plant growth, control of insects on the plant, and/or stress resistance. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in the soil and cultivated using conventional procedures to produce transgenic plants. The transgenic plants are propagated from the planted transgenic seeds under conditions effective to impart disease resistance to plants, to enhance plant growth, to control insects, and/or to impart stress resistance. While not

wishing to be bound by theory, such disease resistance, growth enhancement, insect control, and/or stress resistance may be RNA mediated or may result from expression of the elicitor polypeptide or protein.

When transgenic plants and plant seeds are used in accordance with the present invention, they additionally can be treated with the same materials as are used to treat the plants and seeds to which a hypersensitive response elicitor polypeptide or protein is applied. These other materials, including hypersensitive response elicitors, can be applied to the transgenic plants and plant seeds by the above-noted procedures, including high or low pressure spraying, injection, coating, and immersion. Similarly, after plants have been propagated from the transgenic plant seeds, the plants may be treated with one or more applications of the hypersensitive response elicitor to impart disease resistance, enhance growth, control insects, and/or to impart stress resistance. Such plants may also be treated with conventional plant treatment agents (e.g., insecticides, fertilizers, etc.).

EXAMPLES

Example 1 - Bacterial Strains and Plasmids

Escherichia coli DH5 and BL21 were purchased from Gibco BRL (Rockville, MD) and Novagen (Madison, WI) respectively.

pET28 plasmids were from Novagen (Madison, WI).

All restriction enzymes (e.g., NdeI and HindIII), T4 DNA ligase, Calf intestinal alkaline phosphatase (CIP), and PCR reagents were from Gibco BRL (Rockville, MD).

Oligonucleotides were synthesized by Lofstrand Labs Ltd (Gaithersburg, MD).

Chemically synthesized polypeptides were synthesized by Bio-Synthesis (Lewisville, TX).

Example 2 - Construction of Truncated Gene Encoding Harpin

Fragments of genes encoding harpin proteins were constructed in pET28 vector and expressed in *E. coli* as follows;

1. HrpN fragments were PCR amplified from the pCPP2139 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
2. HrpZ fragments were PCR amplified from the pSYH10 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
3. PopA fragments were PCR amplified from the pBS::popA plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.
4. HrpW fragments were PCR amplified from the pCPP1233 plasmid (Cornell University, Ithaca, NY) and cloned into pET28 vector.

All truncated fragments were amplified by PCR with full length harpin DNA as the template.

Oligonucleotides corresponding to the truncated N-terminal sequence were started /modified with a Nde I site (which serves as an initiation codon of methionine (ATG)). Oligonucleotides corresponding to a C-terminal sequence contained a UAA stop codon followed by a Hind III site.

PCR was carried in a 0.5 ml tube with GeneAmpTM 9600 and 9700 (PE Applied Biosystems, Branchburg, New Jersey). 45 µl of SuperMix^{TM} (Gibco BRL, Rockville, MD) was mixed with 20 pmoles of each pair of DNA primers, 10 ng of full length harpin DNA, and diH_2O to fill the final volume to 50 µl. After heating the mixture at 95°C for 2 min., PCR was performed for 30 cycles at 94°C for 1 min., 58°C for 1 min. and 72°C for 1.5 min. Amplified DNAs were purified with QIAquick PCR purification kit (QIAGEN Inc., Vlencia, CA), digested with Nde I and Hind III at 37°C for 5 hours, extracted once with phenol:chloroform:isoamylalcohol (25:24:1), and precipitated with ethanol. 5 µg of pET28(b) vector DNA was digested with 15 units of Nde I and 20 units of Hind III at 37°C for 3 hours followed with calf intestinal alkaline phosphatase treatment for 30 min. at 37°C to reduce the background resulting from incomplete single enzyme digestion. Digested vector DNA was purified with the QIAquick PCR purification kit and directly used for ligation. Ligation was carried at 14°C for 12 hours in a 15 µl mixture containing about 50 to

100 ng of digested pET28(b), 10 to 30 ng of targeted PCR fragments, and 1 unit of T4 DNA ligase. 5 µl of ligation solution was added to 100 µl of DH5α/XL1-Blue competent cells, placed in 15 ml Falcon tube, and incubated on ice for 30 min. After heat shock at 42°C for 45 seconds, 0.9 ml SOC solution (20 g bacto-tryptone, 5 g bacto-yeast extracts, 0.5 g NaCl, 20 mM glucose in one liter) was added into the tube and incubated at 37°C for 1 hour. 20 µl of transformed cells were plated onto LB agar plate with 30 µg/ml of kanamycin and incubated at 37°C for 14 hours. Single colonies were transferred to 3 ml LB-media and incubated overnight at 37°C. Plasmid DNA was prepared in a 2 ml culture with QIAprep Miniprep kit according to the manufacture's instruction. The DNA sequence of truncated harpin constructions was verified with restriction enzyme analysis and sequencing analysis. Plasmids with the desired DNA sequence were transferred into the BL21 strain with a standard chemical transformation method as indicated above.

Example 3 - Expression of Proteins

A single clone of *E. coli* with a constructed gene was grown overnight at 37°C in LB with kanamycin. A proper amount of overnight culture was transferred to 50 to 500 ml LB and incubated at 37°C until OD600 reached 0.5 to 0.8. ITPG was added to the culture which was further incubated at room temperature for a period of 5 hour to overnight. Alternatively, a proper amount of overnight culture was transferred to 50 to 500 ml of ½ TB with lactose medium (6 g bacto-trypton, 12 g bacto-yeast extract, 75 g lactose in one liter). After incubation at 37°C until the OD600 reached 0.5 to 0.8, the culture was incubated at room temperature for a period of 5 hours to overnight.

All bacterial cells were harvested by centrifugation and resuspended in 1:5 TE buffer (10 mM Tris, pH 8.5 and 1 mM EDTA). The cells were disrupted by sonication and clarified by centrifugation. Supernatants were then infiltrated into tobacco leaves for HR testing.

Heat treatment (i.e. boiling for 1 to 10 min.) was used to achieve further purification.

All truncated fragments of genes encoding harpin protein were expressed in *E. coli*/ BL-21, DE3 strain with an N-terminal His-tag and 20 to 21

amino acid residues generated from the expression vector sequence. The His-tag sequence did not affect the HR activity of the proteins. In some cases, Ni-Agarose beads were added into supernatant solution and mixed at 4°C to room temperature for a period of 30 min. to overnight. The proteins bound to the Ni-Agarose beads were washed by 0.1 M imidazole buffer, and proteins were eluted with 0.6 to 1.0 M imidazole. After dialysis against 10 mM Tris, pH 8.5 buffer, the proteins were infiltrated into tobacco leaves for HR testing.

For proteins expressed in *E. coli* that were difficult to dissolve in water, total cells were resuspended and sonicated in 8 M urea buffer (0.1M Na-phosphate, 10 mM Tris buffer, pH8.0). The total cell lysate was centrifuged, and supernatants were collected. Ni-agarose was added into the supernatants and mixed gently at room temperature for 30 min. The Ni-agarose resin was washed with buffer (8 M urea, 0.1 M Na-phosphate, 10 mM Tris buffer, pH6.3). The proteins were eluted with elution buffer (8 M urea, 0.1 M EDTA, 0.1 M Na-phosphate, 10 mM Tris buffer, pH 6.3) and dialyzed against buffer (pH 8.5, 10 mM Tris) with stepwise decreased urea. If the proteins still were insoluble in buffer, the solution pH was adjusted to 9 to 11 and sonicated at room temperature for 1 to 5 min.

Chemically synthesized polypeptides were dissolved in 10 mM Tris, pH 6.5 to 11 buffers depending on their solubility.

A hypersensitive response ("HR") assay was performed by infiltration of 0.1 to 0.3 ml of serial diluted protein solutions into tobacco leaves (cv. Xanth). All HR data shown in these examples were recorded from 48 hours after infiltration.

Example 4 - Quantification of Proteins

All expressed proteins were checked with pre-cast 4-20% SDS polyacrylamide gel electrophoresis (SDS-PAGE) from Novex (San Diego, CA). After electrophoresis, the gel was stained with Coomasssie R-250 solution (0.1% Coomassie R-250, 10% Acetate Acid, 40% ethanol) for 1 to 4 hours and distained with distaining solution (8% acetate acid and 25% ethanol) overnight. The density of corresponding bands were compared to standard proteins, which were either purchased from Novex or were from quantitative standard harpin protein produced by Eden Bioscience (Bothell, Washington).

Example 5 - Classification of Harpin Proteins

Since harpin proteins share common biochemical and biophysical characteristics as well as biological functions, based on their unique properties, HR elicitors from various pathogenic bacteria should be viewed as belonging to a new protein family—i.e. the harpin protein family. The harpin protein can be classified into at least four subfamilies based on their primary structure and isolated sources. As set forth in Table 1, those subfamilies are identified by the designation N, W, Z, A, etc.

Table 1 - Subfamilies of Harpin Proteins

Harpin proteins	Isolated Source	Classified Subfamily	pI	Amino acids	Heat stable	Core structure
$HrpN_{Ea}$	*E. amylovora*	N	4.42	403	Yes	No
$HrpN_{Ech}$	*E. chrysanthemi*	N	6.51	340	Yes	No
$HrpN_{Ecc}$	*E. carotovora*	N	5.82	356	Yes	No
$HrpN_{Est}$	*E. stewartii*	N	N/A	N/A	Yes	No
$HrpW_{Pss}$	*P. syringae*	W	4.43	424	Yes	No
$HrpW_{Ea}$	*E. amylovora*	W	4.46	447	Yes	No
$HrpZ_{Pss}$	*P. syringae*	Z	3.95	341	Yes	No
PopA1	*R.solanacearum*	A	4.16	344	Yes	No

Example 6 - Analysis of the Structural Units of an HR Domain

The sequence of amino acids that alone could elicit a hypersensitive response in plants (i.e. HR domains) has been investigated in different ways. It was reported that a carboxyl-terminal 148 amino acid portion of $HrpZ_{Pss}$ is sufficient and necessary for HR (He et al., "Pseudomonas Syringae pv. Syringae $Harpin_{pss}$: A Protein that is Secreted via the Hrp Pathway and Elicits the Hypersensitive Response in Plants," Cell 73:1255-1266.(1993), which is hereby incorporated by reference). With truncated HrpZ fragments, it was determined that an N-terminal 109 amino acids and C-terminal 216 amino acids of $HrpZ_{Pss}$, respectively, were found to elicit HR (Alfano et al., "Analysis of the Role of the Pseudomonas Syringae pv. Syringae HrpZ Harpin in Elicitation of the Hypersensitive Response in Tobacco Using

Functionally Non-polar hrpZ Deletion Mutations, Truncated HrpZ Fragments, and hrmA Mutations," Molecular Microbiology 19:715-728 (1996), which is hereby incorporated by reference). Jin et al., "A Truncated Fragment of $Harpin_{pss}$ Induces Systemic Resistance to Xanthomonas campestris pv. Oryzae in Rice," Physiological and Molecular Plant Pathology 51:243-257 (1997), which is hereby incorporated by reference, reported that a truncated $HrpZ_{Pss}$ with an N-terminal of 137 amino acids elicited a hypersensitive response in tobacco and induced systemic acquired resistance (i.e. SAR) in rice. After digestion with protease, a hypersensitive response active fragment of $HrpN_{Ea}$ was isolated and found to span amino acids 137 to 204 of $HrpN_{Ea}$. It was found that a 98 residue of N-terminal $HrpN_{Ea}$ fragment was the smallest bacterially produced peptide that displayed HR-eliciting activity (Laby, "Molecular Studies on Interactions Between Erwinia Amylovora and its Host and Non-host Plants," Doctoral Thesis in Cornell University (1997), which is hereby incorporated by reference).

A series of $HrpN_{Ea}$ fragments have been generated with His-tag fusion at the N-terminal of the polypeptides and a polypeptide ($HrpN_{Ea}137180$), located at position of 137 to 180 amino acid residue of $HrpN_{Ea}$, was identified to elicit HR activity in tobacco.

Example 7 - Analysis of Secondary Structure of HR Domains

The DNA and primary protein sequence of the $HrpN_{Ea}137180$ show no any homologues among other hypersensitive response elicitors.

Analyses of the secondary structure of the fragment of $HrpN_{Ea}137180$ revealed, with the aid of the computer program Clone Manger5 (Scientific & Educational Software, Durham, NC), that there was a beta-form, a beta-turn, and unordered forms. One typical α-helical segment of residues at 157-170 was found in the $HrpN_{Ea}137180$ polypeptide. To determine the function of this structure, polypeptides with a disrupted α-helical structure were generated and hypersensitive response results were evaluated. As shown in Table 2, a complete alpha-helix unit (H unit), probably with a length greater than 12 amino acid residues, is need for hypersensitive response activity.

Table 2 - Effect of Alpha-helix Structure

Fragment name	Amino acid	HR*	Structure	Source
$HrpN_{Ea}137180$	137-180 (44) pI= 3.10	+ <5 μg/ml	Complete H	E.coli expressed peptide
$HrpN_{Ea}137166$	137-166 (30) pI = 3.29	-	disrupted H	Synthesized peptide
$HrpN_{Ea}76168$	76-168 pI = 3.39	-	disrupted H	E.coli expressed peptide

The α-helical unit plays an important role in hypersensitive response activity; however, it was found that an α-helix unit alone did not achieve HR (Table 3).

Therefore, hypersensitive response eliciting domains contain more than one structure unit. Besides the core α-helical unit, there is an acidic unit that has no typical secondary structure feature but is rich in acidic amino acids. This relaxed structure, having a sheet and random turn, is designated as an acidic unit (A unit).

Although the acidic unit is important in achieving a hypersensitive response, it alone, like the α-helical unit alone, did not elicit a hypersensitive response.

A synthetic polypeptide, $HrpN_{Ea}140176$, that included both A and H structure, spanning amino acids 140 to 176 of $HrpN_{Ea}$, gave full activity of HR. Sequence analysis by major search engines revealed no global primary sequence similarity in the databases to $HrpN_{Ea}140176$, even among the harpin protein families.

Table 3 - Effect of Acidic Unit on Hypersensitive Response (HR) Activity

Fragment name	Amino acid	HR*	Structure (A or H)**	Source
$HrpN_{Ea}140176$	140-176 (37) pI=3.17	+ <5 μg/ml	A + H	Synthesized peptide
$HrpN_{Ea}157170$	157-170 (14) pI = 6.94	-	**H**	Synthesized peptide
$HrpN_{Ea}137156$	137-156 (20) pI = 2.67	-	A	Synthesized peptide

Example 8 - Hypersensitive Response Domain Structure of $HrpN_{Ea}$

Four α-helical regions with at least 12 amino acid residues were found in $HrpN_{Ea}$ based on computer analysis with the program Clone Manager 5 (Scientific & Educational Software, Durham, NC), which predicts the secondary structure of protein from the primary sequence by the method of Garnier-Osguthorpe-Robson.

It is believed that a hypersensitive response domain includes two structural units, the α-helix (H) and the acidic unit (A). Another hypersensitive response domain, spanning amino acids 43 to 70 in HrpNEa, was found. A minimal sequence of 12 to 14 AA residues of both the H and A units is believed to be needed. The chemically synthesized polypeptide of $HrpN_{Ea}4370$ gave full HR activity in tobacco. Thus, a second HR domain has been discovered based on purely secondary structure analysis and prediction.

To further test the hypothesis that the A and H units are needed to achieve a hypersensitive response, an approach of unit exchange (i.e. swapping an acidic unit from one HR domain to another HR domain) was designed. A polypeptide of $HrpN_{Ea}$Dswap, which consisted of the acidic unit of a hypersensitive response domain ($HrpN_{Ea}140176$), spanning amino acids 136 to 156 of $HrpN_{Ea}$, and the α-helical unit of another hypersensitive response domain ($HrpN_{Ea}4370$), spanning amino acids 57 to 70 of $HrpN_{Ea}$, was chemically synthesized. This polypeptide swapped two structural units of A and H between two hypersensitive response domains of $HrpN_{Ea}4370$ and $HrpN_{Ea}140176$. The $HrpN_{Ea}$Dswap gave a hypersensitive response activity in tobacco (Table 4). This result shows that the structural characteristic of an HR domain determines its activity, and structural analysis can be used to determine hypersensitive response activity.

Table 4 - Two Structural Units Determine Hypersensitive Response Activity

Fragment name	Amino acid	HR	Structure Type	Source
$HrpN_{Ea}4370$	43-70 (28) pI= 3.09	+ <5 μg/ml	A + H	Synthesized peptide Partial soluble
$HrpN_{Ea}$Dswap	HrpN136156 (A)+ HrpN5770 (H) pI=2.67	<20 μg/ml	A unit from $HrpN_{Ea}140176$ + H unit from $HrpN_{Ea}4370$	Synthesized peptide Partial soluble

Example 9 - Prediction of Hypersensitive Response Domains Among Proteins in Harpin Family

The secondary structure which indicates the presence of a hypersensitive response domain in HrpNEa was used to identify other harpin proteins, including proteins classified as different subfamilies. Structural prediction of a hypersensitive response domain among harpin proteins was carried according to following criteria:

1. There are two structural units in a hypersensitive response domain, including:
   a. A stable α-helix unit with 12 or more amino acids in length and
   b. An hydrophilic, acidic unit with 12 or more amino acids in length which could be a beta-form, a beta-turn, and unordered forms.
2. The pI of a hypersensitive response domain should be acidic and, in general, below 5.
3. The minimal size of an HR domain is from about 28 to 40 AA residues.

Putative HR domains have been identified to fit the criteria by computer analysis among harpin protein family (Table 5).

Table 5 - Predication of Hypersensitive Response Domains Among Harpin Proteins

HR domain	Isolated Source	Predicted region*	pI	Structure
$HrpN_{Ea}$-1	*E. amylovora*	43-70	3.09	A + H
$HrpN_{Ea}$-2	*E. amylovora*	140-176	3.17	A + H
$HrpN_{Ech}$-1	*E. chrysanthemi*	78-118	5.25	A + H
$HrpN_{Ech}$-2	*E. chrysanthemi*	256-295	4.62	A + H
$HrpN_{Ecc}$-1	*E. carotovora*	25-63	4.06	A + H
$HrpN_{Ecc}$-2	*E. carotovora*	101-140	3.00	A + H
$HrpW_{Pss}$-1	*P. syringae*	52-96	4.32	A + H
$HrpW_{Ea}$-1	*E. amylovora*	10-59	4.53	A + H
$HrpZ_{Pss}$-1	*P. syringae*	97-132	3.68	A + H
$HrpZ_{Pss}$-2	*P. syringae*	153-189	3.67	A + H
$HrpZ_{Pss}$-3	*P. syringae*	271-308	3.95	A + H
$PopA1_{Rs}$-1	*R.solanacearum*	92-125	3.75	A + H
$PopA1_{Rs}$-2	*R.solanacearum*	206-260	3.62	A + H

*Amino acid residue position

Example 10 - Hypersensitive Response Activity of Select Synthesized Polypeptides

Polypeptides were produced by expression in either *E. coli* or by chemical synthesis. Based on prediction of solubility and stability of a particular peptide, in some cases, a broader region of AA residues in addition to the essential units were also synthesized to increase solubility of the peptides. The identification of HR domains among four subfamilies of harpin protein demonstrated this (Table 6).

Table 6 - Hypersensitive Response Activity of Select Synthesized Polypeptides

HR domain	Isolated Source	Synthesized region	pI	Source	HR activity
$HrpN_{Ea}$-1	*E. amylovora*	43-70	3.09	Chemical Synthesized	+ < 5 μg/ml
$HrpN_{Ea}$-2	*E. amylovora*	140-176	3.17	Chemical Synthesized	+ < 5 μg/ml
$HrpW_{Ea}$-2	*E. amylovora*	10-59	4.53	E.coli expressed	+ < 5 μg/ml
$HrpZ_{Pss}$-1	*P. syringae*	97-132	3.68	Chemical Synthesized	+ < 20 μg/ml
$HrpZ_{Pss}$-1	*P. syringae*	153-189	3.69	E.coli expressed	+ < 5 μg/ml
$PopA1_{Rs}$-1	*R. solanacearum*	92-125	3.75	Chemical Synthesized	+ < 5 μg/ml
$PopA1_{Rs}$-2	*R. solanacearum*	206-260	3.62	E.coli expressed	+ < 5 μg/ml

Example 11 - Construction of Hypersensitive Response Domains in a Protein Expression Cassette

Polypeptides with a harpin protein hypersensitive response domain were expressed in *E. coli*. PCR was used to amplify desired areas of genes encoding harpin proteins and cloned into an expression vector, e.g. pET28a. A pair of PCR primers with unique flanking sequences were designed to create a universal expression cassette, as shown in Figure 1, for expression of a fragment of harpin protein. Each amplified DNA fragment has a protein translation start codon of ATG in a restriction enzyme Nde I site which might add an extra amino acid of methionine into a polypeptide. Each amplified DNA fragment has a protein translation stop codon of TAA. Each amplified fragment contained two restriction enzyme sites of EcoR V and Sma I, which gave 4 extra in-frame amino acids expressed as Pro-Gly at the N-terminal and Asp-Ile at the C-terminal, respectively. Those two sites are essential to allow two or more expression cassettes to be linked in a specific order and in frame with a minimum number of amino acids being introduced. Cassette A was first digested by EcoR V, ligated to cassette B, and digested with Sma I to produce a new expression cassette C which coupled the two fragments together with two extra amino acids (i.e. Asp-Gly), which are common amino acids in hypersensitive response domains. The newly formed cassette C still contained the same 5' and 3' flanking sequences as original cassettes A and B and maintained the ability to be

coupled by another cassette. Bgl II and Bam HI sites in the cassette permit the cassette to be linked in frame into a cancatomer with a correct orientation. The strategy is that digestion of DNA with Bgl II and Bam HI results in compatible ends that would be ligated with each other but could not be cut by either enzymes after ligation. For example, a DNA fragment encoding a hypersensitive response domain in a cassette could be digested by restrictions enzymes of Bgl II and Bam H1 separately, digested DNA fragments could be ligated in a ligation solution also including both Bgl II and Bam HI enzymes, any ligated ends with Bgl II or Bam HI sites could be digested by the enzymes, and only those ligated sites between Bgl II and Bam HI could remain.

Example 12 - Building Blocks for Creating Superharpins that have Higher Biological Efficacy

Hypersensitive response domains were identified and isolated from several harpin proteins. With the combination of those HR domains, new polypeptides (i.e. superharpins) that have higher HR potency and have enhanced ability to induce disease resistance, impart insect resistance, enhance growth, and achieve environmental stress tolerance. Superharpins could be one HR domain repeat units (cancatomer), different combinations of HR domains, and/or biologically active domains from other elicitors. Part of the domains from different harpin proteins and other elicitors were constructed into the universal expression cassette as shown on Example 11 and designated as superharpin building blocks. Table 7 lists some superharpin building blocks which were expressed in pET-28a(+) vector with a His-tag sequence at their N-terminal.

Table 7 - Superharpin Building Blocks including pET-28a(+) his-tag Leader Sequence

Domain Sequence	Source	MW (kDa)	#a.a.	pI	Soluble	(Structurally) Heat Stable
A	PopA70-146	10.69	104	6.48	Yes	Yes
(N_N)	HrpNEa40-80	6.754	68	6.78	N/A	N/A
$(N_N)_2$	Dimer of HrpNEa40-80	10.84	111	6.13	N/A	N/A
$(N_N)_3$	Triplemer of HrpNEa40-80	14.93	154	5.63	N/A	N/A
$(N_N)_4$	Tetramer of HrpNEa40-80	19.01	197	4.95	N/A	N/A
(N_C)	HrpNEa140-180	7.224	68	5.01	Yes	Yes
$(N_C)_2$	Dimer of HrpNEa140-180	11.78	111	3.98	Yes	Yes
$(N_C)_3$	Triplemer of HrpNEa140-180	16.34	154	3.72	Yes	Yes
$(N_C)_4$	Tetramer of HrpNEa140-180	20.89	197	3.58	Yes	Yes
$(N_C)_{10}$	Cancatomer (10 repeating units of HrpNEa140-180	48.23	455	3.28	N/A	N/A
$(N_C)_{16}$	Cancatomer (16 repeating units of HrpNEa140-180	75.57	713	3.18	N/A	N/A
W	HrpWEa10-59	7.986	77	6.48	N/A	N/A
Z_N	HrpZ90-150	8.087	78	5.38	Yes	Yes
$Z_{266-308}$	HrpZ266-308	7.029	70	6.40	Yes	Yes
his-tag leader seq.		2.045	19	11.04		

<u>Example 13</u> - Superharpins with Stacked HR Domains and their Biological Activities

There are numerous polypeptides could be generated with different combinations of HR domains or by stacking HR domains and repeating units in order. Selective combination or stacking of HR domains isolated from harpin proteins or other elicitors can be designed to achieve a targeted disease resistance spectrum. See Table 8 for superharpins prepared by stacking of HR building blocks listed on Table 7. All three listed superharpins (i.e. SH-1, SH-2, SH-3) were constructed into a pET28(a) vector and expressed in *E. coli*. Recombinant proteins were partially purified and quantified by SDS-PAGE with purified Harpin N protein as a quantitative standard.

Table 8 - Properties of Superharpins

Protein	Domain Sequence	MW (kDa)	# a.a.	pI	Soluble	Heat Stable
SH-1	*$W(N_N)_4A(N_C)_4Z_{266\text{-}308}$	54.955	545	3.69	Yes	Yes
SH-2	*$W(N_N)_4Z_M(N_C)_4Z_{266\text{-}308}$	52.341	519	3.54	Yes	Yes
SH-3	*$W(N_N)_4Z_M(N_C)_4Z_{266\text{-}308}A$	60.375	598	3.67	Yes	Yes
HrpNEa	HrpN from E.amylovora	39.697	403	4.42	Yes	Yes

Bioassays for hypersensitive response on tobacco leaves (HR), percentage of TMV reduction on tobacco leaves, and plant growth enhancement with tomato showed that superharpins had higher (up to 2 to 10 fold greater) HR potency compared with HrpN from *E. amylovora*. This also demonstrated that superharpins have better performance on % TMV reduction and plant growth enhancement assay. See Table 9.

Table 9 - Biological Activities of Superharpins

Protein	Domain Sequence	Elicit HR (~µg/ml)	% TMV reduction on tobacco		% Plant Growth Enhancement	
			10 µg/ml	1 µg/ml	10 µg/ml	1 µg/ml
SH-1	$W(N_N)_4A(N_C)_4Z_{266\text{-}308}$	0.66	83	79	7.49	9.83
SH-2	$W(N_N)_4Z_M(N_C)_4Z_{266\text{-}308}$	0.13	84	60	11.05	7.30
SH-3	$W(N_N)_4Z_M(N_C)_4Z_{266\text{-}308}A$	0.15	77	55	11.07	10.00
HrpNEa	HrpN from E.amylovora	1-3	55	10	11.68	N/A

Although the invention has been described in detail for the purpose of illustration, it is understood that such detail is solely for that purpose, and variations can be made therein by those skilled in the art without departing from the spirit and scope of the invention which is defined by the following claims.